

Interactions Between Oral Streptococci and Oral Fluid Components, with Special Reference to β_2 -Microglobulin.

Dan Ericson



Malmö 1984

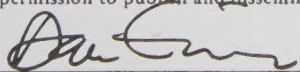
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Title and subtitle INTERACTIONS BETWEEN ORAL STREPTOCOCCI AND ORAL FLUID COMPONENTS, with Special Reference to β_2 -Microglobulin			
Abstract Binding of radiolabelled immunoglobulin (Ig)G, G1, G2, G3, A1, A2, M1 and M2 and haptoglobin, hemoglobin, fibrinogen and aggregated β_2-microglobulin (β_2m) to strains of <u>Streptococcus mutans</u>, <u>S.sanguis</u>, <u>S.mitior</u>, <u>S.milleri</u> and <u>S.salivarius</u>, was investigated. Aggregated β_2m bound to strains of all species tested. IgA1 bound to one strain of <u>S.milleri</u>. The bacterial binding structures for aggregated β_2m were sensitive to pepsin digestion, heat- and formaldehyde treatment. Monomeric β_2m could bind to selected strains of <u>S.mutans</u> in 0.05 M KCl buffer with 1mM $CaCl_2$. In saliva, β_2m was present as monomers. Between 0.2-0.9 $\mu g/ml$ was found in parotid and 0.2-0.6 $\mu g/ml$ in submandibular/sublingual saliva. Gel filtered parotid saliva fractions containing β_2m could agglutinate strains of <u>S.mutans</u> which bound β_2m. Goat anti-human β_2m antiserum could specifically decrease or inhibit the agglutination of β_2m-binding <u>S.mutans</u> strains in parotid saliva. It is suggested that salivary β_2m may contribute to the agglutination of <u>S.mutans</u> strains by parotid saliva. Whole saliva from 4 subjects were incubated with a total of 20 strains of <u>S.mitior</u> and <u>S.salivarius</u> isolated from the saliva donors. In saliva, 6-8 antigens were detected by tandem crossed immunoelectrophoresis against rabbit anti-human saliva antiserum. In homologous saliva/bacteria combinations, bacteria absorbed fewer antigens as compared to what was seen for heterologous combinations. It is suggested that such reactions involve a certain degree of host and/or bacteria specificity.			
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AND ORAL FLUID COMPONENTS,

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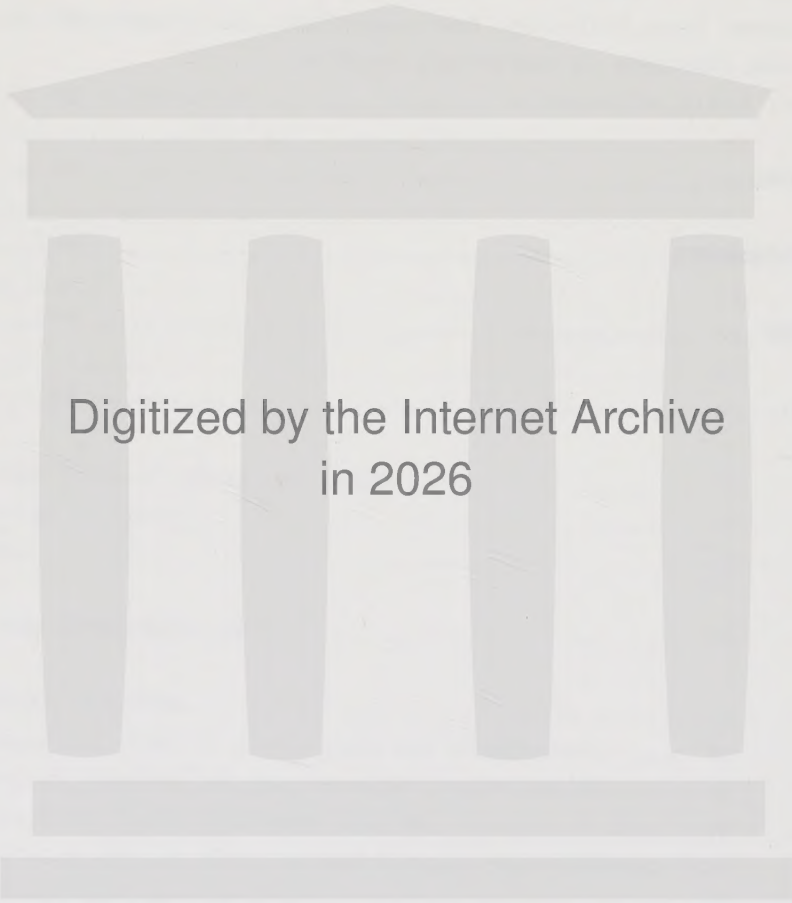
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PREFACE

This thesis is based on the following papers which are referred to by their Roman numerals.

- I Dan Ericson, Douglas Bratthall, Lars Björck, Erling Myhre, and Göran Kronvall. 1979. Interactions between human serum proteins and oral streptococci reveal occurrence of receptors for aggregated β_2 -microglobulin. *Infect. Immun.*, 25:279-283.
- II Dan Ericson, Lars Björck, and Göran Kronvall. 1980. Further characteristics of β_2 -microglobulin binding to oral streptococci. *Infect. Immun.*, 30:117-124.
- III Dan Ericson, Douglas Bratthall, Lars Björck, and Göran Kronvall. 1982. β_2 -microglobulin in saliva and its relation to flow rate in different glands in man. *Archs. Oral Biol.*, 27:679-682.
- IV Dan Ericson. 1984. Agglutination of Streptococcus mutans by low-molecular-weight salivary components: effect of β_2 -microglobulin. *Infect., Immun.* In press, 1984.
- V Dan Ericson. 1984. Salivary interactions with homologous and heterologous strains of oral streptococci and epithelial cells. Submitted for publication.

ABBREVIATIONS

β_2^m	; β_2 -microglobulin.
C_H^3	; A constant heavy chain domain of immunoglobulin having cytophilic properties.
$F(ab')_2$	; The bivalent antigen combining domains of immunoglobulins.
HLA	; Major human histocompatibility antigens.
HMWG	; High molecular weight salivary glycoproteins.
Ig	; Immunoglobulins.

INTRODUCTION

The two major dental diseases, dental caries and periodontitis, are caused by bacterial plaque adhering to tooth surfaces. The initial attachment of microorganisms to the tooth surface is a prerequisite for the development of bacterial plaque and disease.

An extensive number of studies have shown that bacterial attachment in the oral cavity is modified by oral fluid-bacteria interactions (for reviews see Gibbons, 1980, 1984; van Houte, 1982, 1983, 1984). Oral fluid components originating from salivary glands and the gingival crevice are selectively absorbed onto the enamel and form the acquired pellicle to which bacteria attach in a highly selective manner (reviews by Gibbons, 1980, 1984; van Houte, 1982, 1983, 1984).

It has been suggested that lectin-like bacterial adhesins have specific affinity for oral fluid molecules, which are present in the pellicle (receptors). Bacteria may possess multiple adhesins with different specificity (Gibbons et al., 1983). In addition, a variety of phenomena have been considered important for "unspecific" bacterial attachment mechanisms (Staat and Peyton, 1984). Such mechanisms involve hydrophobic interactions (Nesbitt et al., 1982a,b; Gibbons and Etherden, 1983; Rosenberg et al., 1983a,b; Svanberg et al., 1984), van der Waals forces and ionic interactions (reviews by Marshall et al., 1971; Gibbons, 1980; Glantz and Larsson, 1981; Norde 1984).

Since oral fluids are constantly bathing the surfaces to which bacteria attach, certain molecules may competitively inhibit the interactions between bacterial adhesins and acquired pellicle receptors (reviews by Gibbons, 1980, 1984; Ericson et al., 1984). Furthermore, oral fluid components can interact with unattached bacteria and modify their surface properties, sometimes even agglutinating them. The concentration of bacterial agglutinating factors has been proposed to influence the attachment of bacteria; a high concentration in saliva will preferably cause the clumping of bacteria into large aggregates, which are more easily flushed away by saliva and swallowed. A lower concentration may induce the formation of small aggregates and is suggested to enhance attachment (Ørstavik, 1977; Lilje-

mark et al., 1981).

It has been proposed that salivary molecules display microheterogeneities which contribute to the selective attachment or clearance of different members of the oral flora (review by Tabak et al., 1982). Salivary molecules can express individual variations (review by Arglebe, 1981), and Streptococcus mutans strains have been reported to attach in higher numbers to homologous than to heterologous pellicles (Ørstavik et al., 1982). Thus, the question of whether bacteria are able of recognizing subject-specific molecular variants might be raised.

The complex nature of the attachment mechanisms in the mouth is further underlined by the fact that salivary molecules with bacterial-binding properties can interact with each other (Eggert, 1980; Olsson et al., 1981; Prakobphol et al., 1982; Shomers et al., 1982; Reinholdt and Kilian, 1984).

The pellicle components can be enzymatically altered, thus changing the bacterias ability to colonize the pellicle (Gibbons and Etherden, 1982). The enzymatic degradation of salivary glycoproteins can result in a loss of their carbohydrates and less specific bacterial agglutination mediated by the protein cores, which has been proposed to increase bacterial accumulation once the pioneer bacteria have colonized the aquired pellicle (Leach and Agalamanyi, 1984). Immunoglobulin A1 (IgA1) proteases from oral streptococci can degrade IgA1-containing secretory IgA. This might give enzyme-producing strains an ecological advantage (Kilian and Holmgren, 1981; Kilian et al., 1980).

The effect of bacterial interaction with an individual molecular species in saliva is very complex to evaluate. Nevertheless, it is of great importance to gain more information about the interactions between bacteria and specific saliva components and the combined effect of them, in an attempt to understand the selective attachment and phenomenon occurring in the mouth (review by van Houte, 1984).

The purpose of this thesis is to obtain further information about bacterial-binding substances in oral fluids. Evidence is presented that β_2 -microglobulin and a number of other oral fluid substances can interact with oral indigenous bacteria.

ORAL FLUID COMPONENTS AND BACTERIA

The oral fluids contain substances originating from salivary glands, gingival crevices, bacteria, epithelial cells and tooth surfaces. A large number of these substances have the potential to interact with bacteria; a few of these have been extensively studied. In the following pages the most studied and so far most interesting substances related to bacterial agglutination and adhesion in the oral cavity are discussed.

HOST DERIVED SUBSTANCES

High molecular weight salivary glycoproteins (HMWG)

Glycoproteins are proteins with covalently linked oligosaccharide units. The carbohydrate content is often 60-70% and galactose, fucose, mannose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid are abundant. HMWG have been ascribed various biological functions, (for a review see Tabak et al., 1982) such as being a selective permeability barrier which guards the mucous membranes against desiccation and mechanical insult, a lubricant and a fluid vehicle with antimicrobial activity.

Gibbons and Spinell (1970) first reported that high molecular weight salivary molecules could induce agglutination of plaque bacteria. They found that the agglutinating activity depended on the presence of calcium ions. Hay et al. (1971) showed that the isolated agglutinating substance was a high molecular weight glycoprotein. Ericson et al. (1976) isolated a glycoprotein (MW 10^6 - 10^7) from parotid saliva which agglutinated a S. mutans serotype c strain. HMWGs which can agglutinate certain oral bacteria are also adsorbed onto hydroxyapatite (Hay et al., 1971; Ericson and Magnusson, 1976; Kondo et al., 1976; Ørstavik, 1978; Ericson et al., 1984).

Although several species of oral bacteria are agglutinated by HMWG, some degree of bacterial specificity of these molecules has been described (Kashket and Donaldsson, 1972; Ericson et al., 1973; Ericson et al., 1976; Ericson and Magnusson, 1976; Kashket and Hankin, 1977; Levine et al., 1978; Hogg and Embery, 1979; Rundegren and Ericson, 1981a). HMWG can also possess blood group reactivity (Gibbons and Qureshi, 1978; Kondo et al., 1978; Hogg and Embery, 1979, 1982). The blood group reactive substances have been suggested to inhibit the attachment of oral bacteria to human buccal epithelial cells, probably by mimicking cell-bound receptors (Williams and Gibbons, 1975; Slots and Gibbons, 1978). Sugar residues and sialic acid on the macromolecules seem to be important for interaction with bacteria (McBride and Gisslow, 1977; Ericson et al., 1976; Kondo et al., 1978; Levine et al., 1978; Gibbons and Qureshi, 1979; Qureshi and Gibbons, 1981).

Calcium

Calcium is present in saliva at a concentration of approximately 1 mM and calcium can be bound to salivary macromolecules (Lagerlöf and Lindqvist, 1982). The presence of calcium is important for the bacterial agglutinating effect of some high molecular weight glycoproteins (Gibbons and Spinell, 1970; Hay et al., 1971; Kashket and Donaldson, 1972; Hogg and Embery, 1979; Rundegren and Ericson, 1981b). Calcium has been implicated in adherence and cohesion in plaque by bridging between negatively charged molecules on bacteria and salivary molecules (Rölla, 1980; Tatevossian, 1981).

Immunoglobulins

Immunoglobulins of the immunoglobulin classes IgA, IgG and IgM are present in oral fluids (Tomasi and Ziegelbaum, 1963; Lehner et al., 1967; Brandtzaeg et al., 1970; Oon and Lee, 1972; review by Hanson and Brandtzaeg, 1980). Secretory IgA is the major immunoglobulin in saliva and consists of two monomeric IgA molecules linked together with a polypeptide called joining chain (Halpern and Koshland, 1970) and a glycoprotein called secretory component (for a review see Hanson and Brandtzaeg, 1980).

The presence of the secretory component has been proposed to make secretory IgA more resistant to degradation by enzymes than serum IgA

(Ghetie and Mota, 1973; Lindh, 1975; Underdown and Dorrington, 1974). However, strains of oral streptococci can produce IgA1 protease (Plaut et al., 1974; Kilian and Holmgren, 1981), which can cleave secretory IgA (Kilian et al., 1980). It has been further suggested that the relative resistance of secretory IgA to cleavage by IgA proteases might be due to the presence of IgA1 protease-neutralizing secretory IgA antibodies.

Salivary IgA antibodies can bind to plaque bacteria (Brandtzaeg et al., 1968) and agglutinate them, thereby inhibiting their adherence to human buccal epithelial cells (Williams and Gibbons, 1972). Agglutination of bacteria by salivary IgA may be enhanced by the binding of the immunoglobulin to HMWG (Eggert, 1980; Olsson et al., 1981; Reinholdt and Kilian, 1984). Removal of S. sanguis-agglutinating IgA from saliva has been found to decrease agglutination as well as adherence to saliva-coated hydroxyapatite (Liljemark et al., 1979). They concluded that S. sanguis could utilize salivary IgA in the pellicle to colonize tooth surfaces. In contrast, Genco and Taubman (1973) proposed that salivary IgA may prevent adhesion of S. mutans to the teeth. The dual effect of IgA on bacterial adherence was further studied by Kilian et al. (1981) who found that specific IgA antibodies increased the attachment of S. sanguis to hydroxyapatite, but decreased attachment of S. mutans, S. mitior and S. salivarius.

The major contribution of IgG and IgM antibodies in saliva is via the crevicular fluid, which also contains a number of other serum components (Brill and Krasse, 1958; Brandtzaeg, 1965; Brandtzaeg et al., 1970; Attström et al., 1975). Immunization attempts against S. mutans have evoked IgG-antibody responses and caries reductions (review by Brandtzaeg, 1984).

Fibronectin

Fibronectin is a glycoprotein with a molecular weight of approximately 450.000 daltons (for a review see Ruoslahti, 1981), and is present in saliva and gingival crevice material (Babu et al., 1983; Bratthall et al., 1983). Fibronectin binds to collagen and to cell surfaces and is considered to be a "tissue glue". Fibronectin also has properties of a nonspecific opsonin, which could facilitate phagocytosis of tissue debris.

Fibronectin can bind to the surface of Staphylococcus aureus (Kuusela, 1978; Mosher and Proctor, 1980; Doran and Reynor, 1981), group A, C and G streptococci (Simpson et al., 1982; Myhre and Kuusela, 1983) and to strains of oral streptococci (Babu et al., 1983). The presence of fibronectin on epithelial cell surfaces has been suggested to promote attachment of S. pyogenes to the cell surfaces (Simpson and Beachey, 1983). Proteases in saliva may degrade fibronectin, especially when oral hygiene is poor (Etherden and Gibbons, 1984).

Babu et al. (1983) showed that oral streptococcal strains could be agglutinated by fibronectin. Binding of fibronectin to hydroxyapatite has also been demonstrated (Babu et al., 1983) as well as its presence in artificial salivary pellicles. However, pretreatment of hydroxyapatite with saliva supplemented with fibronectin did not significantly alter the adhesion of S. mutans and S. sanguis strains tested (Lamberts et al., 1984).

α -Amylase

α -amylase is a low molecular weight (approximately 5.5×10^4) enzyme present in saliva (review by Jacobsen et al., 1972). The enzyme splits the α -1,4 glucosidic bonds of glucan, thus splitting starches into smaller saccharides. The enzyme can interact with oral streptococci and agglutinate strains of S. sanguis (Douglas, 1983) and S. mutans (Sönju et al., 1984). Douglas (1983) found that bacterial binding of the enzyme did not correlate with their attachment to saliva-coated hydroxyapatite. He suggested that the enzyme might concentrate products of amylolytic action close to the cell.

Lysozyme

Lysozyme is a low molecular weight ($1.5-1.7 \times 10^4$) (Balekjian et al., 1969) enzyme present in saliva and other body fluids (van Palenstein Helderman, 1976; Skurk et al., 1979). Lysozyme has affinity for the murein peptidoglycan of bacterial cell walls (Salton, 1961) and breaks the glycosidic β -1,4 bond between N-acetylmuramic acid and N-acetylglucosamine. Lysozyme can agglutinate oral streptococci (Pollock et al., 1976). Even if the enzymatic active site of the enzyme is blocked, aggregation of S. mutans can be promoted. Lysozyme can also inhibit growth of S. mutans without lysis of the bacterium (Iacono et al., 1980), and it is suggested that binding of lysozyme

to bacteria may activate autolytic enzymes (Goodman et al., 1981). Lysozyme has also been reported to interact with other salivary substances (Shomers et al., 1982), which might modify the bacterial-lysozyme interactions.

Salivary lipids

Saliva contains glycolipids, triglycerides and free fatty acids (Slomiany et al., 1980, 1981). Fatty acids are sometimes covalently bound to glycoproteins (Slomiany et al., 1984a). Lipids are thus present in the enamel pellicle (Slomiany et al., 1984b). Saliva from caries resistant persons were found to contain glycolipids and phospholipids that bound to hydroxyapatite better than did the lipids from caries susceptible persons (Jozwiak et al., 1984). Gibbons and Etherden (1983) showed that highly hydrophobic strains of S. sanguis and Actinomyces viscosus adsorbed similarly to pellicles formed by untreated saliva and by saliva depleted of free lipids. Lipid depleted pellicles showed higher number of binding sites for S. mutans, which could suggest that lipids may have a protective function (review by Gibbons, 1984). This finding agrees with the higher concentration of certain lipids in pellicles formed from caries resistant saliva (Jozwiak et al., 1984). In addition, since salivary glycoproteins may contain covalently bound lipids, these domains can promote unspecific hydrophobic interaction with certain bacteria (Gibbons, 1984).

β_2 -microglobulin (β_2m)

β_2m was first identified by Berggård in 1964 as a low molecular weight (11.815) urinary protein. Berggård and Bearn (1968) further reported on its physico-chemical properties. β_2m has 100 amino acid residues (MW 11.815), and consists of a single polypeptide with two half-cystinyl residues involved in a disulfide bond. β_2m is devoid of carbohydrates (Berggård and Bearn, 1968; Cunningham et al., 1973), and has an isoelectric point of 5.8 (Berggård et al., 1980). It was found that the peptide sequence of β_2m was similar to that of immunoglobulin domains, in particular the C_H3 domain of IgG (Peterson et al., 1972).

β_2m is present on the cell surface of lymphocytes and other cell types (Peterson et al., 1974; Nilsson et al., 1973, 1974) and possib-

ly also arranged as aggregates (Fesus et al., 1981). β_2m is non-covalently associated with the human major histocompatibility complex (HLA) as its light chain (Grey et al., 1973; Nakamuro et al., 1973; Peterson et al., 1974) and β_2m is associated with the major histocompatibility complex in several other animal species (review by Berggård et al., 1980). A portion of β_2m on cells may exist unbound to HLA (Solheim and Torsby, 1974).

β_2m is released from the cell surfaces by secretion and by shedding during cell membrane turnover (Nilsson et al., 1974). Subsequently β_2m is found as monomers in all normal biological fluids, including saliva (Ervin et al., 1971; Talal et al., 1975; Plesner and Bjerrum, 1980). A small part of the β_2m in serum is associated with HLA complexes (Plesner and Bjerrum, 1980). Its concentration in whole saliva is approximately 1.1 ± 0.55 mg/l (Ervin et al., 1971), and in parotid saliva 0.84 ± 0.20 mg/l (Talal et al., 1975). Talal et al. (1975) observed high levels of β_2m in the saliva of patients with Sjögren's syndrome and rheumatoid arthritis. Elevated levels of β_2m in serum have been associated with increased cellular turnover, as in malignancy and inflammation (for reviews see Karlsson et al., 1980; Plesner, 1980).

Talal et al. (1975) suggested that the elevated levels of β_2m in saliva of Sjögren's and rheumatoid patients were due to local production and not passive leakage from serum, since higher levels were found in saliva. High levels of β_2m in serum do not increase the salivary output (review by Revillard, 1979). No correlation between salivary, colostrum and serum levels of β_2m was found by Ervin et al. (1971).

The function of β_2m is still uncertain. Data summarized by Berggård et al. (1980) suggest that β_2m is involved in immunological recognition phenomena between cells and in cell differentiation.

Non-immune bacterial binding of immunoglobulin Fc-portion has been described for staphylococci, (Forsgren and Sjöqvist, 1966) and later for streptococci of group A, C and G (Kronvall, 1973). The biochemical similarity between β_2m and Ig domains raises the possibility of an analogous binding of β_2m to bacteria. Indeed, aggregated β_2m ,

above molecular weight 100,000, has been found to bind to group A, B, C and G streptococci (Kronvall et al., 1978; Schönbeck et al., 1981), but β_2m is bound to other binding structures than those binding Ig. However, β_2m in its monomeric form neither binds to these bacteria nor competitively inhibits the binding of the aggregated β_2m (Kronvall et al., 1978, 1979a).

It has been suggested that the aggregated form of β_2m could be significant for binding bacteria to cell surfaces, since β_2m may be available there in the form of repetitive units, functionally similar to the arrangement of β_2m in aggregates. In fact, attachment of group A streptococci to buccal epithelial cells has been specifically inhibited with anti- β_2m F(ab')₂ fragments supporting this contention (Björk et al., 1982). Moreover, parasites can bind β_2m and Schistosoma mansoni is able to bind unaggregated human β_2m (Torpier et al., 1979). Binding of β_2m and other host molecules by this parasite might help to evade the immune response by mimicking the host (reviews by Bloom, 1979; Greenblatt, 1983).

Since β_2m is present in saliva and is able to bind to various species of streptococci, an investigation into its interaction with oral streptococcal surfaces and its possible binding to other salivary macromolecules is warranted.

SUBSTANCES FROM BACTERIA AND FOOD

Bacterial polymers

Extracellular glucans from oral streptococci can agglutinate bacteria and are considered important for plaque formation (for review see Hamada and Slade, 1980). It has been suggested that glucans increase bacterial cohesion in dental plaque and contribute to the initial adherence of some microorganisms to the pellicle. However, in the presence of saliva, glucan synthesis by S. mutans has no effect upon the initial attachment of bacteria to saliva-coated hydroxyapatite (Clark and Gibbons, 1977). Clark et al. (1978) found that glucans did not promote attachment of S. mutans to saliva-coated hydroxyapatite. In contrast, Röllä et al. (1983a,b) have shown that human saliva contains free glucosyl- and fructosyl-transferases which can synthe-

size water-insoluble polysaccharides and that glucosyltransferase can adsorb to saliva treated hydroxyapatite. It was proposed that glucosyltransferase in the pellicle is capable of synthesizing glucans to which glucan-coated S. mutans cells can attach. Other bacterial polymers has been suggested to affect bacterial colonization such as dextrans from other plaque bacteria and heteropolysaccharides from A. viscosus (for review, see van Houte, 1982). However, recently it was shown by Ellen and co-workers (1983) that fresh isolates of A. viscosus and A. naeslundii seldom were agglutinated by polysaccharides. They concluded that plaque polysaccharides were not likely to be responsible for Actinomyces cohesive interactions in plaque.

Lectins

Lectins are proteins which bind to specific carbohydrates in polysaccharides or glycoconjugates (Lis and Sharon, 1973; Sharon and Lis, 1975; Nachbar and Oppenheim, 1980). Recently, Gibbons and Dankers (1983) demonstrated that components of the human diet contain lectins which can bind to both bacterial and host surfaces. In their study, various lectins persisted on the surface of bacteria and epithelial cells for several hours after ingestion and also increased the attachment of a S. sanguis strain. They proposed that the ingestion of similar lectins in foods could affect bacterial colonization, as also suggested by Bratthall (1978).

AIMS OF THE INVESTIGATIONS

- * To study interactions between a number of different proteins present in serum or oral fluids and oral streptococci.
- * To study some physico-chemical properties of the β_2 -microglobulin binding sites on oral streptococci and their distribution among streptococcal species.
- * To determine the concentration of β_2 -microglobulin in saliva and its relationship to salivary flow rate and to other salivary molecules.
- * To study the effect of the binding of β_2 -microglobulin to Streptococcus mutans with special reference to bacterial agglutination.
- * To study interactions between oral fluid antigens detected by tandem crossed immunoelectrophoresis and homologous and heterologous strains of oral streptococci.

EXPERIMENTAL RESULTS

Bacterial binding of radiolabelled substances

In Papers I and II the interaction between bacteria and radiolabelled proteins and glycoproteins was investigated. Briefly, radioactive iodine (^{125}I) was incorporated into the test molecule. Bacterial suspensions were then mixed with a small amount of radiolabelled protein. After washing and centrifugation, the radioactivity of the bacterial pellets was expressed as a per cent of the added radioactivity.

All experiments were performed with bacteria suspended in phosphate buffered saline (PBS), pH 7.2 with 0.02 % sodium azide. All protein solutions contained Tween 20 (0.05%) or human serum albumin (0.2%) to prevent unwanted absorption to the vessel walls.

Thirteen radiolabelled human proteins were screened for interactions with 31 strains of oral streptococci representing S. mutans, S. salivarius, S. sanguis, S. mitior and S. milleri. Human immunoglobulins (Ig) polyclonal G, monoclonal G1, G2, G3, G4, A2, M1 and M2 and hemoglobin, haptoglobin and fibrinogen did not bind to the bacteria tested. Significant amounts of aggregated $\beta_2\text{m}$ bound to 20 strains and myeloma IgA1 showed affinity for one strain of S. milleri.

Binding of aggregated $\beta_2\text{m}$ by oral streptococci

The discovery of oral streptococcal $\beta_2\text{m}$ binding initiated an extended investigation of some physico-chemical properties and of the occurrence of the binding site among 85 strains of oral streptococci (Paper II). In this series of experiments, approximately one third of the strains, representing all species tested were found to bind aggregated $\beta_2\text{m}$. As a group, S. sanguis group I:A strains were exceptions by not binding the protein. Among the binding strains, there were no obvious species or serotype pattern.

Influence of ionic strength and pH

The presence of saliva increased the binding of aggregated $\beta_2\text{m}$ to strains of oral streptococci more than twofold, as compared to a control group A streptococcus strain. Therefore, the binding of aggregated $\beta_2\text{m}$ to oral streptococci was studied in the presence of

various buffers to evaluate the influence of ionic strength and pH. When 77 strains were tested for binding in 0.066 M PBS, pH 7.2, strains of S. milleri and S. salivarius in particular showed an increased binding as compared to in 0.15 M PBS. Three of five S. milleri and 2 of 11 S. salivarius bound aggregated β_2m in 0.15 M PBS. In 0.066 M PBS, all S. milleri and 9 of 10 S. salivarius strains bound aggregated β_2m .

At least four different patterns of salt concentration-dependent affinity for aggregated β_2m were seen. S. sanguis and S. milleri strains showed binding maxima at approximately 0.03 M, which rather rapidly decreased when more salt was added. S. mutans strains showed maxima at 0.08 M, which decreased slowly when more salt was added. The negative Staphylococcus strains did not bind aggregated β_2m at any salt concentrations tested. The positive group A streptococcus, however, showed a binding maximum at 0.15 M.

At a pH below 6, the binding of aggregated β_2m increased, and below pH 5.5, up to 90 % was bound to bacteria.

Characteristics of oral streptococcal β_2m -binding

The bacterial binding sites for aggregated β_2m on selected strains from all five species were resistant to trypsin treatment (Paper I). Although fibrinogen is known to block the binding of aggregated β_2m to group A, C and G streptococci (Kronvall et al., 1979b), it neither bound to, nor interfered with binding to oral streptococci (Paper II). Pepsin digestion, formaldehyde treatment and heating of bacteria to 50°C for 10 minutes destroyed the binding sites for aggregated β_2m . Radiobelled monomers of β_2m did not bind to the strains tested, nor did unlabelled monomers inhibit binding of the radiolabelled aggregates.

The Scatchard plot of the binding data for the binding of aggregated β_2m to bacteria showed a biphasic curve, indicating a heterogeneity in the binding reactions. Control experiments revealed no binding of aggregated α_1 -microglobulin.

The possible occurrence of β_2m in a functionally aggregated configuration in saliva was not studied extensively. Therefore it was

necessary to determine the form of β_2m and its concentration in saliva (Paper III).

β_2m in saliva

Slightly stimulated parotid saliva from 7 healthy subjects contained 0.2 - 0.9 μg β_2m per ml and from 13 hypogammaglobulinaemic subjects 0.2 - 1.1 $\mu g/ml$ (Paper III). Submandibular/sublingual saliva from 4 healthy subjects contained 0.2 - 0.6 $\mu g/ml$. β_2m -concentrations in parotid saliva were negatively correlated with salivary flow rate, but the opposite was seen for submandibular/sublingual saliva.

Gel filtration of parotid saliva and of a mixture of separately collected parotid/submandibular/sublingual saliva on Sephadex G-200 columns showed that β_2m eluted as monomers. High molecular weight complexes containing β_2m , thus mimicking aggregates of β_2m were not found.

Since the earlier established binding of aggregated β_2m (Papers I, II) was highly influenced by ionic strength, a change of buffer composition towards a more oral physiological one (Burnett and Sherp, 1962), might reveal binding of β_2m monomers (Paper IV).

Binding of monomeric β_2m

In the presence of calcium it was shown that radiolabelled monomeric human β_2m bound to selected strains of S. mutans (Paper IV) which in earlier experiments (Papers I, II) had only bound the aggregated form of β_2m . Monomeric β_2m also bound to one strain which previously had exhibited a variable binding capacity. However, Tween 20 and human albumin had to be excluded from the reaction mixtures for binding to occur. The exclusion of these substances also caused higher adsorption of β_2m to the plastic tubes, but β_2m -binding and non-binding strains still differed clearly.

Agglutination by monomeric β_2m

It became clear that monomers of β_2m could bind to some S. mutans strains under certain conditions, but could β_2m monomer binding also account for the agglutination of these strains? If calcium was present in the buffer solution, purified monomeric human β_2m could agglutinate S. mutans cells. Aggregated β_2m could agglutinate

these strains also in PBS (Paper IV).

Differences in agglutination between β_2 m-binding and non-binding strains were not seen in unfractionated parotid saliva, or in void volume fractions of saliva gelfiltered on Sephadex G-75 columns. Agglutination differences were however noted in β_2 m-containing fractions. 5 of 6 β_2 m-binding strains were agglutinated. The one aberrant strain was agglutinated occasionally, whereas 4 of 5 non-binding strains failed to agglutinate, and the remaining strain showed agglutination in only one of two replications. In void volume fractions, devoid of β_2 m, but rich in IgA and HMWG, agglutination of all strains occurred. Agglutination in these fractions was more rapid than in β_2 m-containing fractions.

Since lysozyme has a molecular weight similar to β_2 m and might be expected to elute close to β_2 m from filtration gels, agglutination by this enzyme was also tested. All strains tested were agglutinated by lysozyme, and there were no systematic differences between β_2 m-binding and non-binding strains.

Effect of anti- β_2 m on agglutination

To investigate whether β_2 m could be of any significance for agglutination of S. mutans in unfractionated parotid saliva, goat anti-human β_2 m antiserum and preimmune serum were added in various concentrations to parotid saliva (Paper IV). To be able to compare the effect of antiserum and control serum simultaneously, an altered method for turbidimetric measurement of bacterial agglutination was developed, as compared to the method according to Ericson et al. (1975). Test cuvettes with anti- β_2 m antiserum were placed in the reference position in the spectrophotometer and the control cuvettes containing preimmune serum were placed in the sample position. Thus, the recorder displayed the starting optical density (OD) as zero if both cuvettes were of the exact density. If the suspension in the reference positioned cuvette agglutinated but not the suspension in sample cuvette, the resulting OD would increase, indicating that the antibodies inhibited agglutination.

Of the 8 S. mutans strains tested, the agglutination of the 5 β_2 m-binding strains was impaired when anti- β_2 m antiserum was added. The

agglutination of 2 negative strains and 1 strain that had shown variable affinity for β_2m was not affected.

Bacterial binding of oral fluid substances from different hosts

In Paper V another method for studying the bacterial binding of salivary molecules was applied. Briefly, a total of 20 oral streptococcal strains isolated from four subjects were mixed with whole clarified saliva from the same subjects in cross-over assays. The remaining antigens in the saliva supernatants after incubation with bacteria were compared with the antigens in control saliva using tandem crossed immunoelectrophoresis assays against rabbit anti-human saliva antiserum.

In bacteria-treated saliva supernatants, up to 7 of the 8 detected antigens were decreased in concentration as compared to the control saliva. The combinations of homologous strains with homologous saliva resulted in the least percentage of interactions, compared with other combinations. Thus, homologous bacteria absorbed fewer of the 6 to 8 detected antigens from homologous saliva as compared to what was seen for other bacteria/saliva combinations. Washed oral epithelial cells also showed fewer interactions with homologous saliva. Commercial hydroxyapatite interacted with all detected antigens.

GENERAL DISCUSSION

This series of papers has demonstrated that oral streptococci can interact with host substances from oral fluids. The interaction between β_2m and bacteria that has been studied, indicates that β_2m can bind to and agglutinate certain strains of oral streptococci. The concentration of β_2m in saliva was found to be sufficient to contribute to salivary induced agglutination of the β_2m -binding S. mutans strains tested. Fresh isolates of S. mitior and S. salivarius strains interacted with a number of oral fluid substances. Fewer oral fluid substances bound to homologous bacteria as compared to heterologous bacteria.

Binding structures for β_2m on oral streptococci

Aggregated β_2m appeared to interact with specific binding sites on the oral streptococci as determined from the Scatchard plot (Paper II). The biphasic slope of the Scatchard plot indicated a heterogeneity in the binding reactions (Dahlquist, 1978). This appearance of Scatchard plots may be a result of differences in affinity of the bacterial binding structures, of a negative cooperativity or of an interaction between bacterial binding sites and partly denatured macromolecules. A lower number of saturable binding sites appeared to exist on bacteria, and also a higher number of binding sites with low affinity. The presence of saturable binding sites indicated a specific interaction. A specificity of the interaction was further indicated by the fact that α_1 -microglobulin, aggregated in the same way as β_2m , did not bind to bacteria.

However, as for group A, C and G streptococci (Kronvall et al., 1978, 1979a), monomers of β_2m were not able to inhibit binding of the aggregated form, nor did radiolabelled monomers bind under the conditions described in Paper II. The radiolabelling procedure might have damaged the site(s) on β_2m important for bacterial binding. Thus, only aggregates containing intact and iodinated β_2m -molecules would bind to bacteria (Kronvall et al., 1978). A multi-point attachment between β_2m and bacterial binding sites seems to be important for a detectable interaction under these circumstances. The possible repetitive arrangement of β_2m on cell surfaces (Fesus et al., 1981), functionally similar to β_2m aggregates, might then be favourable for

bacterial attachment. Supporting this concept, Björck et al. (1982) showed that blocking of cell surface β_2m impaired the attachment of group A streptococci to human buccal epithelial cells.

The physico-chemical properties tested of β_2m binding sites of the oral strains were different from those of group A, C and G streptococci studied by Kronvall et al. (1978, 1979a,b). The marked differences in heat resistance, trypsin digestion sensitivity, fibrinogen inhibition and salt concentration-dependence imply that major differences exist among the binding structures on these strains. In group A streptococci, several different data suggest that aggregated β_2m interact with the M-protein (Björck et al., 1981, 1984). However, the binding of β_2m to other bacterial surface proteins is not excluded (Björck et al., 1984).

The pepsin digestion and the formaldehyde sensitivity of the oral strains indicate the involvement of a protein component in the binding structures carried by these strains. The extreme heat lability of the binding structure on bacteria is not easily explained (Paper II). A similar heat lability has been reported for the bacterial binding sites responsible for salivary induced agglutination of S. sanguis strains (Golub et al., 1979).

The salt concentration dependence seen might reflect the colonization sites of the oral strains, since they exhibited a maximum binding of aggregated β_2m at a salt concentration similar to that in saliva (Burnett and Sherp 1962). The group A streptococcus strains showed a binding maximum in a salt concentration similar to that in serum. This suggests that binding of aggregated β_2m by the oral strains is likely to occur in an oral milieu. The increased binding of aggregated β_2m at a low pH might be of interest in the oral cavity, where the pH can be low.

β_2m in saliva

In Paper III it was found that the β_2m concentrations in saliva were within the lower range of concentrations reported earlier (Evrin et al., 1971; Talal et al., 1975). In parotid saliva, β_2m -concentrations were inversely correlated to secretion rate, in contrast to what had been found by Talal et al. (1975). They described elevated

levels of β_2m in parotid saliva from patients with Sjögren's syndrome. Treatment of the patients resulted in an increased secretion rate and a decreased β_2m concentration. No correlation between flow rate and β_2m concentration in 2 control subjects was found. However, in the present study with 5 persons, the β_2m -concentrations were inversely correlated with flow rate. The decrease of β_2m in patients after treatment of Sjögren's syndrome might have partially been the result of an increased rate of flow.

The positive correlation between flow rate and β_2m concentration in submandibular/sublingual saliva can perhaps be explained by the different secretion processes in parotid and submandibular/sublingual glands. In the latter, the secretion process of the mucous cells is partly apocrine, at least when highly stimulated (Poldermans and de Lange, 1981). Even with normal secretion rate, parts of the cell membranes are shed and excreted with saliva (Tandler and Poulsen, 1976). As β_2m is present on cell membranes, the positive correlation between secretion rate and β_2m concentration might be ascribed to the shedding of β_2m along with parts of cell membranes.

Binding of β_2m -monomers - influence of buffers

Binding structures for monomers of β_2m are likely to exist if the binding of β_2m aggregates is specific, even if the radiolabelled monomers are bound with an extremely low affinity. Since β_2m is present as monomers in saliva (Paper III), a possible role for salivary β_2m was looked for. The salt concentration dependence indicated that binding of monomers to oral indigenous streptococci might be revealed under conditions more like an oral physiological environment. In a buffer solution with a salt composition similar to that in saliva (Burnett and Sherrp, 1962; Gibbons, 1980), including 1 mM of $CaCl_2$, it was found that monomers of β_2m could bind to strains of S. mutans. The importance of calcium for certain bacteria-saliva interactions as seen in this study has been previously well established (Gibbons and Spinell, 1970; Hay et al., 1971; Kashket and Donaldson, 1972; Hogg and Embery, 1979; Eggert, 1980; Rundegren and Ericson, 1981b). The mechanism for the involvement of calcium in the interactions between S. mutans and β_2m is not known. Perhaps calcium might contribute to a formation of weak aggregates of β_2m in the buffer or on the streptococcal surface, thereby providing

conditions for a multi-point interaction. It should however be noted that no aggregates were found in gel filtered saliva fractions.

The binding of radiolabelled β_2m monomers to bacteria was not of high affinity, since it was inhibited by Tween 20 or albumin in concentrations used for the assays with aggregated β_2m (Papers I,II). Nevertheless, monomers of β_2m could agglutinate strains of S. mutans which bound radiolabelled β_2m . Perhaps the unlabelled β_2m bound with a higher affinity than the radiolabelled molecules. This could be of importance for salivary induced agglutination of bacteria, provided that other salivary components, similar to albumin, did not interfere with this binding. The agglutination of β_2m -binding S. mutans strains in β_2m -containing fractions of saliva implied that β_2m monomers could induce agglutination in the presence of some salivary molecules. Other bacterial agglutinins such as IgA or HMWG were not present in these fractions.

Even though the hen egg white lysozyme standard eluted prior to β_2m , small amounts of lysozyme might have been present in β_2m -rich fractions. However, the agglutination of the strains did not correlate with their agglutination by lysozyme. A function for β_2m in the agglutination phenomenon in whole saliva is conceivable, since unfractionated parotid saliva contained more than 10 times the concentration of β_2m needed for agglutination (Paper III).

Void volume fractions containing IgA and HMWG agglutinated all strains at a faster rate than did the β_2m -rich fractions. This suggests that the bacterial agglutinating factors in these fractions were stronger and/or more concentrated. The possible significance of the contribution of β_2m to bacterial agglutination by saliva must thus be compared with the role of these agglutinating factors.

Inhibition of S. mutans agglutination in parotid saliva

Addition of an antiserum against β_2m to unfractionated parotid saliva selectively decreased the agglutination of β_2m -binding S. mutans strains, proposing that β_2m actually contributed to agglutination (Paper IV). The agglutination of the strain with the highest β_2m binding was, however, totally inhibited. This somewhat unexpected finding might have been caused by the adsorption of antigen-antibody

complexes onto the surfaces of the bacterial cells, thus sterically inhibiting the binding of other agglutinating factors. Another explanation to the total inhibition of this strain might be that serum components, aside from antibodies, interfered with other salivary agglutinins or were absorbed onto the bacterial surface. Agglutinins other than β_2m could then have been blocked.

It has been explained that several serum components can interfere with the agglutination phenomenon in saliva (Ericson et al., 1979; Malamud et al., 1984). Consequently, when using whole antiserum as in the present study, the inhibiting effect of other serum components than antibodies was not of interest per se. In the method used, the cuvet arrangement allowed the spectrophotometer to detect only the differences in agglutination between antiserum-containing suspensions and preimmune serum suspensions. Small differences could then be detected, as agglutination in test and control suspensions were measured simultaneously.

Significance of β_2m -binding by oral streptococci

Based on the present findings, it is suggested that β_2m may contribute to the salivary induced agglutination of β_2m -binding S.mutans strains. The magnitude of this contribution is not known.

The binding of β_2m monomers by bacteria is not as strong as the binding of aggregates. Thus, monomers in saliva would probably not interfere with binding of repetitive units of β_2m by direct competition. The higher rate of binding of repetitive units could be of significance for attachment to epithelial cells. This should also be considered for mechanisms affecting the bacterial attachment to tooth pellicles, since it is known that β_2m can bind to hydroxyapatite (Curry et al., 1981). Nevertheless, bacterial agglutination in saliva by β_2m and other salivary agglutinins can increase the disposal of bacteria from the oral cavity and thus influence the attachment and the subsequent colonization as discussed extensively by several authors (Gibbons, 1980, 1984; Tabak et al., 1982; Ericson et al., 1984; van Houte, 1984).

Differences in bacterial binding of oral fluid substances from different hosts

In Paper V fresh bacterial isolates interacted with up to 7 antigens present in saliva. This high degree of interaction contrasts to what was found in Paper I, where few substances interacted with the laboratory strains. Aside from the likelihood that the bacteria did not carry binding sites for these substances, other factors could have influenced the number of detected interactions. The buffer in Paper I might have been unfavourable for revealing of lower affinity interactions, as seemed to be the case for β_2m monomers. It cannot be excluded that bacterial enzymes degraded some salivary substances (Gibbons and Etherden, 1982; Kilian and Holmgren, 1981) in experiments performed in Paper V, thus contributing to the depletion of antigens. Since antigens could be eluted from bacteria with 1 M NaCl after repeated washings with buffer, degradation of these substances was not likely a major cause of their depletion. Aggregation of the substances, other than β_2m in Paper I, might have revealed binding.

When comparing supernatants mixed with bacteria or epithelial cells, at least one antigen always remained in the same concentration in absorbed and control saliva (Paper V). The dilution of the samples had apparently not given a false positive absorption. Hydroxyapatite absorbed all detected substances from saliva and a control as above was not possible.

The lower number of interactions seen between epithelial cells and homologous saliva, as compared to heterologous combinations, might in part be explained by the presence of identical antigens on the cell surfaces and in saliva. The antigens might have been part of the cell surface or absorbed from the saliva and remained after repeated washings, thereby competitively inhibited the binding. The lower number of interactions between salivary antigens and homologous bacteria was probably not due to previously absorbed substances, since the bacteria had been transferred several times in different media.

The findings in Paper V demonstrate that indigenous oral bacteria may interact differently with oral fluid components from different individuals. An involvement of a certain degree of subject/ bacteria

specificity in these interactions is conceivable. The lesser degree of interaction between host substances and homologous bacteria has not to my knowledge been reported earlier.

Inter-individual variations in salivary molecules (Arglebe, 1981) and in pellicle composition (Kraus et al., 1973) have been described. It has also been reported that gut bacteria in piglets can interact selectively with host molecules showing inter-individual differences (Bijlsma et al., 1982). Parallel specificities in host-bacterial interactions are conceivable in the oral cavity. Indeed, Ørstavik et al. (1982) showed that S. mutans strains attached better to pellicles formed by homologous than heterologous saliva.

The findings in the present study apparently contrast at first to those of Ørstavik et al. (1982). Their findings favour the idea that a high affinity for homologous substances are advantageous for colonization. However, this depends upon if these substances are soluble, as in saliva, or if they are present in the pellicle, or both. A high affinity for salivary substances present in the pellicle can increase adhesion, but a high affinity for these components if they are also soluble in saliva may have the opposite effect (reviews by Gibbons, 1980, 1984; van Houte, 1983, 1984; Ericson et al., 1984). Thus, not only bacterial binding of host substances but also "avoidance" of binding might be of importance for bacterial colonization as also proposed by Tabak et al. (1982). The reason for the apparent selective interaction with host substances is not known. Perhaps it reflects an adaptation of the homologous strains (Ørstavik et al., 1982; Ørstavik, 1984) or is a prerequisite for colonization in the first place. Further investigations in this field might eventually explain the significance of these findings.

Some implications of bacterial binding of oral fluid substances

The existence of multiple adhesins on indigenous bacteria would appear to be important for their persistence in the oral cavity by diminishing the unfavourable influence of bacterial surface changes (van Houte, 1982), or binding of host substances interfering with adhesins. Even though multiple adhesins are present on bacteria, binding of small molecules, including various sugars, has been reported to strongly inhibit the attachment of oral bacteria (Kondo et

al., 1976; Liljemark et al., 1978; Gibbons and Qureshi, 1979; Qureshi and Gibbons, 1981). The bacterial binding of soluble host molecules also appearing as receptors on surfaces might thus interfere with adherence (reviews by Gibbons, 1980, 1984).

However, if the primary binding affinity of the soluble molecules is low, as for β_2m for example, such inhibition may not occur. If the same substance appears as a repetitive unit in the pellicle or on cell surfaces, the soluble monomeric substances would be less likely to compete with the higher affinity of such a multi-point attachment. In that case, a large number of low affinity adhesins would not appear unfavourable for attachment.

Further, binding of host molecules by bacteria can change their physico-chemical properties (Miörner et al., 1980). It has been suggested that such interactions make the bacterial surfaces more hydrophilic and less able to interact with important host surface receptors (review by Tabak et al., 1982). Binding of host molecules such as blood group substances, β_2m and IgG by parasites may render them less recognizable by the immune system (reviews by Bloom, 1979; Greenblatt, 1983). This aspect should also be considered for indigenous host-bacterial interactions in the oral cavity.

To the previous knowledge on host-bacterial interactions in the oral cavity, the results in this series of investigations have added oral streptococcal binding of β_2m . Even though the mechanism for β_2m -binding by oral bacteria is not clear, the importance of this interaction for agglutination of S. mutans strains by saliva has been indicated. In the light of the multifarious host-bacterial interactions in the mouth, the significance of this single-type interaction is difficult to evaluate.

A reflection of the high number of host-bacterial interactions in the mouth was presented in Paper V. The lower number of interactions between oral fluid components and indigenous homologous bacteria, as compared with heterologous bacterial strains, reflects the colonization pattern of the strains. A certain degree of specificity in such host-bacterial interactions might therefore be involved in the

mechanisms regulating the colonization of the oral cavity. The significance of these apparently specific interactions is an interesting object for future investigation.

CONCLUSIONS

Interactions between some oral fluid components and oral streptococci have been studied. The main results and conclusions are:

- * Many but not all strains of S. mutans, S. salivarius, S. sanguis, S. mitior and S. milleri can bind aggregated human β_2m .
- * The binding of aggregated and monomeric β_2m is highly influenced by buffer conditions. A salt composition similar to that of saliva promotes binding of both aggregated and monomeric β_2m to oral streptococci.
- * The concentration of β_2m in parotid saliva ranges from 0.2 to 0.9 $\mu g/ml$ and in submandibular saliva from 0.2 to 0.6 $\mu g/ml$.
- * The concentration of β_2m is positively correlated to flow rate in submandibular/sublingual saliva and negatively correlated to flow rate in parotid saliva. β_2m elutes as monomers during various gelfiltration procedures and is seemingly not bound to any other salivary component.
- * Salivary fractions containing β_2m , as well as purified monomers and aggregates of β_2m , could agglutinate β_2m -binding strain of S. mutans. The agglutination by β_2m monomers was dependent on the presence of calcium ions.
- * β_2m in unfractionated parotid saliva is suggested to contribute to the agglutination of β_2m -binding, but not of non-binding S. mutans strains.
- * Indigenous strains of S. salivarius and S. mitior bound less of the detected oral fluid antigens from homologous than from heterologous oral fluids. This might indicate a certain degree of host and/or bacteria specificity in the interactions between indigenous bacteria and host substances.

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LITERATURE CITED

- Arglebe, C. 1981. Biochemistry of human saliva. In: Sialoadenosis and sialoadenitis. Eds: Pfaltz C.R., and R. Chilla. Adv. Oto-Rhino-Laryng., Basel, Karger, 26:97-234.
- Attström, R., A-B. Laurell, U. Larsson, and A. Sjöholm. 1975. Complement factors in gingival crevice material from healthy and inflamed gingiva in humans. J. Periodontal Res., 10:19-27.
- Babu, J.P., W.A. Simpson, H.S. Courtney, and E.H. Beachey. 1983. Interaction of human plasma fibronectin with cariogenic and non-cariogenic oral streptococci. Infect. Immun., 41:162-168.
- Balekjian, A.Y., K.C. Hoerman, and V.J. Berzinskas. 1969. Lysozyme of the human parotid gland secretion: its purification and physico-chemical properties. Biochem. Biophys. Res. Comm., 35:887-894.
- Berggård, I. and A.G. Bearn. 1968. Isolation and properties of a low molecular weight β_2 -globulin occurring in human biological fluids. J. biol. Chem., 243:4095-4103.
- Berggård, B., L. Björck, R. Cigén, and L. Lögdberg. 1980. β_2 -microglobulin. Scand. J. clin. Lab. Invest., 40, Suppl. 154:13-25.
- Bijlsma, I.G.W., A. de Nijs, C. van der Meer, and J.F. Frik. 1982. Different pig phenotypes affect adherence of Escherichia coli to jejunal brush borders by K88ab, K88ac or K88ad antigen. Infect. Immun., 37:891-894.
- Björck, L., H. Miörner, O. Kühnemund, G. Kronvall, and R. Sundler. 1984. On the interaction between β_2 -microglobulin and group A streptococci. Scand. J. Immunol., 20:69-79.
- Björck, L., S. Tylewska, T. Wadström, M. Söderström, and G. Kronvall. 1982. β_2 -microglobulin and its relation to streptococcal M protein and adherence. In: Basic concepts of streptococci and streptococcal diseases. Eds: Holm, S.E. and P. Christenssen. Readbooks Ltd., Chertsey, pp 220-222.
- Björck, L., S.K. Tylewska, T. Wadström, and G. Kronvall. 1981. β_2 -microglobulin is bound to streptococcal M protein. Scand. J. Immunol., 13:391-394.
- Bloom, B.R. 1979. Games parasites play: how parasites evade immune surveillance. Nature, 279:21-26.
- Brandtzaeg, P. 1965. Immunochemical comparison of proteins in human gingival pocket fluid, serum and saliva. Archs. Oral Biol., 10:795-803.
- Brandtzaeg, P. 1984. Role of antibodies in saliva: facts and extrapolations. In: Cariology today. Ed: B. Guggenheim. Int. Congr., Zürich 1983, pp 89-97.
- Brandtzaeg, P., I. Fjellanger, and S.T. Gjeruldsen. 1968. Adsorption of immunoglobulin A onto oral bacteria in vivo. J. Bacteriol., 96:242-249.

Brandtzaeg, P., I. Fjellanger, and S.T. Gjeruldsen. 1970. Human secretory immunoglobulins. I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins. *Scand. J. Heamatol. Suppl.*, 12:1-83

Bratthall, D. 1978. *Daucus carota* (Carrot) - a selective bacteriosorbent. In: *Secretory immunity and infection*. Eds: McGhee J.R., J. Mestecky, and J.L. Babb. Plenum Publ. Corporation, pp 327-333.

Bratthall, G.T., D. Ericson, O. Sandahl, and H.M. Araujo. 1984. Fibronectin in saliva and gingival crevices. *J. Dent. Res.*, 63: Abstract no. 99.

Brill, N. and B. Krasse. 1958. The passage of tissue fluid into the clinically healthy gingival pocket. *Acta Odontol. Scand.*, 16:233-245.

Burnett, G.W. and H.W. Sherp. 1962. *Oral microbiology and infectious diseases*; 2 ed., Williams and Wilkins Co., Baltimore, p 309.

Clark, W. B., L.L. Bammann, and R.J. Gibbons. 1978. Ability of *Streptococcus mutans* and a glucosyltransferase-defective mutant to colonize rodents and attach to hydroxyapatite surfaces. *Infect. Immun.*, 21:681-684.

Clark, W.B. and R.J. Gibbons. 1977. Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of *Streptococcus mutans* 6715 to hydroxyapatite surfaces. *Infect. Immun.*, 18:514-523.

Cunningham, B.A., J.L. Wang, I. Berggård, and P.A. Peterson. 1973. The complete amino acid sequence of β_2 -microglobulin. *Biochemistry*, 12:4811-4822.

Curry, R., J. Thöen and R.P. Messner. 1981. Rapid semi-quantitative isolation of Beta-2-Microglobulin from urine. *J. Immunol. Methods*, 47:365-373.

Dahlqvist, F.W. 1978. The meaning of Scatchard and Hill plots. *Methods Enzymol.*, 48:270-299.

Doran, J.E. and R.H. Raynor. 1981. Fibronectin binding to protein A-containing staphylococci. *Infect. Immun.*, 33:683-689.

Douglas, C.W.I. 1983. The binding of human salivary α -amylase by oral strains of streptococcal bacteria. *Archs. Oral Biol.*, 28:567-573.

Eggert, F.M. 1980. The nature of secretory agglutinins and aggregating factors. IV. Complexing between non-mucin glyco-proteins, immunoglobulins and mucins in human saliva and amniotic fluid. *Int. Archs. Allergy appl. Immun.*, 62:46-58.

Ellen, R.P.E., E.D. Fillery, and K. Seckington. 1983. Infrequent polysaccharide cohesion among *Actinomyces viscosus* and *Actinomyces naeslundii* fresh isolates of human origin. *J.Periodont.Res.*, 18:50-55.

Ericson, Th., D. Bratthall, and J. Rundegren. 1979. Bacterial agglutination induced by saliva. Inhibition studies with anti-Ig antisera and serum components. In: *Saliva and dental caries*. Eds: Kleinberg, I, S.A. Ellison and, I.D. Mandel. Sp. Suppl. Microbiol. Abstr., pp 243-254.

Ericson, Th., A. Carlén, and E. Dagerskog. 1976. Salivary aggregating factors. In: Microbial aspects of dental caries. Eds: Stiles, H.M., W.J. Loesche and T.C. O'Brien. Sp.Supp. Microbiol. Abstr. pp 151-162.

Ericson, Th. and I. Magnusson. 1976. Affinity for hydroxy-apatite of salivary substances inducing aggregation of oral streptococci. Caries Res., 10:8-18.

Ericson, Th., J. Olsson, W. Bowen, J. Ciardi, and J. Rundegren. 1984. Effect of a purified human salivary agglutinin on the adsorption of *S. mutans* to hydroxyapatite. In: Bacterial adhesion and preventive dentistry. Eds: ten Cate, J.M., S.A. Leach, and J. Arends. IRL Press Ltd (Oxford), pp 63-71.

Ericson, Th., K. Pruitt, and D. Bratthall. 1973. On the specificity of bacterial aggregation. J. Dent. Res., 52 Abstract no. 585.

Ericson, Th., K. Pruitt, and H. Wedel. 1975. The reaction of salivary substances with bacteria. J. Oral Pathol., 4:307-323.

Etherden, I., and R.J. Gibbons. 1984. Association of fibronectin - degrading enzymes in saliva with oral hygiene. J. Dent. Res. 63; Special issue. Abstract no. 1359.

Evrin, P.-E., P.A. Peterson, L. Wide, and I. Berggård. 1971. Radio-immunoassay of β_2 -microglobulin in human biological fluids. Scand. J. clin. Lab. Invest., 28:439-443.

Fesus, L., A. Falus, A. Erdei, and K. Laki. 1981. Human β_2 -microglobulin is a substrate of tissue transglutaminase: polymerisation in solution and on the cell surface. J. Cell Biol., 89:706-710.

Forsgren, A. and J. Sjöqvist. 1966. Protein A from *Staphylococcus aureus*. I. Pseudo-immune reaction with human γ -globulin. J. Immunol., 97:822-827.

Genco, R.J. and M.A. Taubman. 1973. Biological properties of secretory IgA antibodies. In: Comparative immunology of the oral cavity. Eds: Mergenhausen, S.E. and H.W. Scherp. U.S. Government Printing Office, Washington, D.C. p.25-37.

Ghetie, V. and G. Mota. 1973. The decrease of human colostral immunoglobulin A resistance to papain action after gradual release of the secretory component. Immunochemistry, 10:839-844.

Gibbons, R.J. 1980. Adhesion of bacteria to surfaces in the mouth. In: Microbial adhesion to surfaces. Eds: Berkeley, R.C.W., J.M. Lynch, J. Melling, P.R. Rutter, and B. Vincent. Soc. Chem. Indust., Chichester, England: Ellis Horwood Ltd., pp 351-388.

Gibbons, R.J. 1984. Adherent interactions which may affect microbial ecology in the mouth. J. Dent. Res., 63:378-385.

Gibbons, R.J. and Dankers I. 1983. Association of food lectins with human oral epithelial cells in vivo. Archs. Oral Biol., 28:561-566.

Gibbons, R.J., and I. Etherden. 1982. Enzymatic modification of bacterial receptors on saliva treated hydroxyapatite surfaces. Infect. Immun., 36:52-58.

Gibbons, R.J. and I. Etherden. 1983. Comparative hydrophobicities of oral bacteria and their adherence to salivary pellicles. *Infect. Immun.*, 41:1190-1196.

Gibbons, R.J., E.C. Moreno, and I. Etherden. 1983. Concentration-dependent multiple binding sites on saliva-treated hydroxyapatite for Streptococcus sanguis. *Infect. Immun.*, 39:280-289.

Gibbons, R.J. and J.V. Qureshi. 1978. Selective binding of blood group-reactive salivary mucins by Streptococcus mutans and other oral organisms. *Infect. Immun.*, 22:665-671.

Gibbons, R.J. and J.V. Qureshi. 1979. Inhibition of adsorption of Streptococcus mutans strains to saliva-treated hydroxyapatite by galactose and certain amines. *Infect. Immun.*, 26:1214-1217.

Gibbons, R.J. and D.M. Spinell. 1970. Salivary-induced aggregation of plaque bacteria. In: Dental plaque. Ed.: Mc Hugh, D., Livingstone Ltd, Edinburgh, England, pp 207-216.

Glantz, P-O. and K. Larsson. 1981. On surface charge and intraoral adhesion. In: Tooth surface interactions and preventive dentistry. Eds. Rölla, G., T. Sönju, and G. Embery. IRL Press (London), pp 181-191.

Golub, E.E., M. Thaler, C. Davis, and D. Malamud. 1979. Bacterial aggregating activity in human saliva: simultaneous determination of free and bound cells. *Infect. Immun.*, 26: 1028-1034.

Goodman, H., J.J. Pollock, V.J. Iacono, W. Wong, and G.D. Shockman. 1981. Peptidoglycan loss during hen egg white lysozyme - inorganic salt lysis of Streptococcus mutans. *J. Bacteriol.*, 146:755-763.

Greenblatt, C.L. 1983. Molecular mimicry and the carbohydrate language of parasitism. *ASM News*, 49:488-493.

Grey, H.M., R.T. Kubo, S.M. Colon, M.D. Poulik, P. Cresswell, T. Springer, M. Turner, and J.L. Strominger. 1973. The small subunit of HL-A antigens is β_2 -microglobulin. *J. exp. Med.*, 138:1608-1612.

Halpern, M.S. and M.E. Koshland. 1970. Novel subunit in secretory IgA. *Nature*, 228:1276-1278.

Hamada, S. and H.D. Slade. 1980. Biology, immunology, and cariogenicity of Streptococcus mutans. *Microbiol. Rev.*, 44:331-384.

Hanson, L.Å. and P. Brandtzaeg. 1980. The mucosal defense system. In: Immunologic disorders in infants and children. Eds: Stiehm, E.R. and V.A. Fulginiti. W.B. Saunders, 2 ed., Philadelphia, pp 137-164.

Hay, D.I. 1973. The interaction of human parotid salivary proteins with hydroxyapatite. *Archs. Oral Biol.*, 18:1517-1529.

Hay, D.I., R.J. Gibbons, and D.M. Spinell. 1971. Characteristics of some high molecular weight constituents with bacterial aggregating activity from whole saliva and dental plaque. *Caries Res.*, 5:111-123.

Hogg, S.D. and G. Embery. 1979. The isolation and partial characterization of a sulphated glycoprotein from human whole saliva which aggregates strains of Streptococcus sanguis but not Streptococcus mutans. Archs. Oral Biol., 24:791-797.

Hogg, S.D. and G. Embery. 1982. Blood-group-reactive glycoprotein from human saliva interacts with lipoteichoic acid on the surface of Streptococcus sanguis cells. Archs. Oral Biol., 27:261-268.

van Houte, J. 1982. Bacterial adherence and dental plaque formation. Infection, 10: 252-260.

van Houte, J. 1983. Bacterial adherence in the mouth. Rev. Infect. Dis., 5, Suppl. 4; 659-669.

van Houte, J. 1984. Are the concepts proposed for dental plaque formation valid?. In: Cariology today. Ed: B. Guggenheim. Int. Congr. Zürich. 1983, pp. 182-190.

Iacono, V.J., B.J. MacKay, S. DiRienzo, and J.J. Pollock. 1980. Selective antibacterial properties of lysozyme for oral bacteria. Infect. Immun., 29:623-632.

Jacobsen, N., K.L. Melvaer, and A. Hensten-Pettersen. 1972. Some properties of salivary amylase: a survey of the literature and some observations. J. Dent. Res., Suppl. to no. 2, 51:381-388.

Jozwiak, Z., B.L. Slomiany, A. Slomiany, V.L.N. Murty, K. Petropoulos, and I.D. Mandel. 1984. Interaction of salivary lipids with hydroxyapatite. J. Dent. Res., 63; Special Issue. Abstract no. 904.

Karlsson, F.A., L. Wibell, and P.E. Evrin. 1980. β_2 -microglobulin in clinical medicine. Scand. J. Clin. Lab. Invest., 40, Suppl., 154: 27-37.

Kashket, S. and C.G. Donaldson. 1972. Saliva-induced aggregation of oral streptococci. J. Bacteriol., 112:1127-1133.

Kashket, S. and S.R. Hankin. 1977. Interaction of streptococcal aggregating factors with thiol- and disulphide-reactive compounds. Archs. Oral Biol., 22:49-53.

Kilian, M. and K. Holmgren. 1981. Ecology and nature of immunoglobulin A1 protease-producing streptococci in the human oral cavity and pharynx. Infect. Immun., 31:868-873.

Kilian, M., J. Mestecky, R. Kulhavy, M. Tomana, and W.T. Butler. 1980. IgA1 proteases from Haemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitidis, and Streptococcus sanguis: comparative immunochemical studies. J. Immunol., 124:2596-2600.

Kilian, M., K. Roland, and J. Mestecky. 1981. Interference of secretory immunoglobulin A with sorption of oral bacteria to hydroxyapatite. Infect. Immun., 31:942-951.

Kondo, W., M. Sato, and H. Ozawa. 1976. Haemagglutinating activity of Leptotrichia buccalis cells and their adherence to saliva-coated enamel powder. Archs. Oral Biol., 21:363-369.

Kondo, W., M. Sato, and N. Sato. 1978. Properties of the human salivary aggregating factor for Leptotrichia buccalis cells. Archs. Oral Biol., 23:453-458.

Kraus, F.W., D. Ørstavik, D.C Hurst, and C.H. Cook. 1973. The aquired pellicle: variability and subject-dependence of specific proteins. J. Oral Pathol., 2:165-173.

Kronvall, G. 1973. A surface component in group A, C, and G streptococci with non-immune reactivity for immunoglobulin G. J. Immunol., 111:1401-1406.

Kronvall, G., L. Björck, E.B. Myhre, and L.W. Wannamaker. 1979a. Binding of aggregated β_2 -microglobulin, IgG, IgA, and fibrinogen to group -A, -C and -G streptococci, with special reference to streptococcal M protein. In: Pathogenic streptococci. Proc VIIth Int. Symp. on streptococci and streptococcal diseases. Sept. 1978. Ed.: M.T. Parker, Reedbooks Ltd., Chertsey, England, pp 74-76.

Kronvall, G., E.B. Myhre, L. Björck, and I. Berggård. 1978. Binding of aggregated human β_2 -microglobulin to surface protein structure in group A, C, and G streptococci. Infect. Immun., 22:136-142.

Kronvall, G., C. Schönbeck, and E. Myhre. 1979b. Fibrinogen binding structures in β -hemolytic streptococci group A, C, and G. Comparison with receptors for IgG and aggregated β_2 -microglobulin. Acta path. microbiol.scand., 87:303-310.

Kuusela, P. 1978. Fibronectin binds to Staphylococcus aureus. Nature, London, 276:718-720.

Lagerlöf, F. and L. Lindqvist. 1982. A method for determining concentrations of calcium complexes in human parotid saliva by gel filtration. Archs. Oral Biol., 27:735-738.

Lamberts, B.L., E.D. Pederson, L.G. Simonson, and B.R. Merrell. 1984. Effect of fibronectin on binding of S. mutans and S. sanguis to hydroxyapatite. J. Dent. Res., 63: Special issue. Abstract no. 159.

Leach, S.A. and E.A. Agalamanyi. 1984. Hydrophobic interactions that may be involved in the formation of dental plaque. In: Bacterial adhesion and preventive dentistry. Eds: ten Cate, J.M., S.A Leach, and J. Arends. IRL Press Ltd (Oxford), pp 43-50.

Lehner, T., J.E. Cardwell, and E.D. Clarry. 1967. Immunoglobulins in saliva and serum in dental caries. Lancet, 1:1294-1297.

Levine, M.J., M.C. Herzberg, M.S. Levine, S.A. Ellison, M.W. Stinson, H.C. Li, and T. van Dyke. 1978. Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycoproteins with Streptococcus sanguis and Streptococcus mutans. Infect. Immun., 19:107-115.

Liljemark, W.F., C.G. Bloomquist, and G.R. Germaine. 1981. Effect of bacterial aggregation on the adherence of oral streptococci to hydroxyapatite. Infect. Immun., 31:935-941.

Liljemark, W.F., C.G. Bloomquist, and J.C. Ofstehage. 1979. Aggregation and adherence of Streptococcus sanguis: role of human salivary immunoglobulin A. Infect. Immun., 26:1104-1110.

Liljemark, W.F., S.V. Schauer, and C.G. Bloomquist. 1978. Compounds which affect adherence of Streptococcus sanguis and Streptococcus mutans to hydroxyapatite. J. Dent. Res., 57:373-379.

Lindh, E. 1975. Increased resistance of immunoglobulin A dimers to proteolytic degradation after binding of secretory component. J. Immunol., 114:284-286.

Lis, H. and N. Sharon. 1973. The biochemistry of plant lectins (phytohemagglutinins). Ann. Rev. Biochem., 42: 541-574.

Magnusson, I. and Th. Ericson. 1976. Effect of salivary agglutinins on reactions between hydroxyapatite and a sero-type c strain of Streptococcus mutans. Caries Res., 10:273-286.

Malamud, D., C. Brown, and R. Goldman. 1984. Inhibition of bacterial aggregation by serum- and blood-derived proteins. Infect. Immun., 43:386-390.

Marshall, K.C., R. Stout, and R. Mitchell. 1971. Mechanisms of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol., 68:337-348.

McBride, B.C. and Gisslow, M.T. 1977. Role of sialic acid in saliva-induced aggregation of Streptococcus sanguis. Infect. Immun., 18: 35-40.

Miörner, H., E. Myhre, L. Björck, and G. Kronvall. 1980. Effect of specific binding of human albumin, fibrinogen, and immunoglobulin G on surface characteristics of bacterial strains as revealed by partition experiments in polymer phase systems. Infect. Immun., 29: 879-885.

Mosher, D.F. and R.A. Proctor. 1980. Binding and factor XIIIa-mediated cross-linking of a 27-kilodalton fragment of fibronectin to Staphylococcus aureus. Science, 209:927-929.

Myhre, E.B. and P. Kuusela. 1983. Binding of human fibronectin to group A, C, and G streptococci. Infect. Immun., 40:29-34.

Nachbar, M.S. and J.D. Oppenheim. 1980. Lectins in the United States diet: a survey of lectins in commonly consumed foods and a review of the literature. Am. J. Clin. Nutr., 33:2338-2345.

Nakamuro, K., N. Tanigaki, and D. Pressman. 1973. Multiple common properties of human β_2 -microglobulin and the common portion fragment derived from HL-A antigen molecules. Proc. Nat. Acad. Sci. USA, 70: 2863-2865.

Nesbitt, W.E., R.J. Doyle, and K.G. Taylor. 1982a. Hydrophobic interactions and the adherence of Streptococcus sanguis to hydroxyapatite. Infect. Immun., 38:637-644.

Nesbitt, W.E., R.J. Doyle, K.G. Taylor, R.H. Staat, and R.R. Arnold. 1982b. Positive cooperativity in the binding of Streptococcus sanguis to hydroxyapatite. Infect. Immun., 35:157-165.

Nilsson, K., P.-E. Evrin, I. Berggård, and J. Pontén. 1973. Involvement of lymphoid and non-lymphoid cells in the production of β_2 -microglobulin - a homologue of the constant domains of IgG. *Nature, London*, 244:44-45.

Nilsson, K., P.E. Evrin, and K.I. Welsh. 1974. Production of β_2 -microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplant Rev.*, 21:53-84.

Norde, W. 1984. Adsorption of biopolymers and its relevance for particle adhesion: a physico-chemical approach. In: *Bacterial adhesion and preventive dentistry*. Eds: ten Cate, J.M., S.A. Leach, and J. Arends. IRL Press Ltd (Oxford), pp 1-17.

Olsson, J., D. Bratthall, and A. Carlén. 1981. Association between bacterial agglutinins and immunoglobulin A in human saliva. *Acta Odontol. Scand.*, 39:61-66.

Oon, C.H. and J. Lee. 1972. A controlled quantitative study of parotid salivary secretory IgA-globulin in normal adults. *J. Immunol. Methods*, 2:45-48.

Ørstavik, D. Sorption of streptococci to glass: effects of macromolecular solutes. *Acta path. microbiol. scand. Sect. B* 85: 47-53.

Ørstavik, D. 1978. The in-vitro attachment of an oral streptococcal sp. to the acquired tooth enamel pellicle. *Archs. Oral Biol.*, 23:167-173.

Ørstavik, D. 1984. Initial bacterial adhesion to surfaces: ecological implications in dental plaque formation. In: *Bacterial adhesion and preventive dentistry*. Eds: ten Cate, J.M., S.A. Leach, and J. Arends. IRL Press Ltd (Oxford), pp 153-166.

Ørstavik, J., D. Ørstavik, and J. Kommisar. 1982. Preferential affinity of oral bacteria for homologous salivary films on dental materials. *Acta Odontol. Scand.*, 40:49-56.

van Palenstein Helderman, W.H. 1976. Lysozyme concentrations in the gingival crevice and at other oral sites in human subjects with and without gingivitis. *Archs. Oral Biol.*, 21:251-255.

Peterson, P.A., B.A. Cunningham, I. Berggård, and G.M. Edelman. 1972. β_2 -microglobulin - a free immunoglobulin domain. *Proc. Natl. Acad. Sci. USA*, 69:1697-1701.

Peterson, P.A., L. Rask, and J.B. Lindblom. 1974. Highly purified papain-solubilized HL-A antigens contain β_2 -microglobulin. *Proc. Natl. Acad. Sci. USA*, 71:35-39.

Plaut, A.G., R.J. Genco, and T.B. Tomasi, Jr. 1974. Isolation of an enzyme which specifically cleaves human immunoglobulin A. *J. Immunol.*, 113:289-291.

Plesner, T. 1980. Immunochemical studies of human β_2 -microglobulin. *Allergy*, 35:627-637.

Plesner, T. and O.J. Bjerrum. 1980. Distribution of "free" and HLA-associated human β_2 -microglobulin in some plasma membranes and biological fluids. *Scand. J. Immunol.*, 11:341-351.

Poldermans, J.E. and G.L. de Lange. 1981. Morphology of secreting serous and mucous sublingual gland cells of the mouse. In: Ed. T. Zelles. *Saliva and salivation. Adv. Physiol. Sci.* 28:147-152.

Pollock, J.J., V.J. Iacono, H. Goodman Bicker, B.J. MacKay, L.I. Katona, L.B. Taichman, and E. Thomas. 1976. The binding, aggregation, and lytic properties of lysozyme. In: *Microbial aspects of dental caries*. Eds: Stiles, H.M., W.K. Loesche, and T.C. O'Brien. *Sp. Supp. Microbiol. Abstracts.* II: 325-352.

Prakobphol, A., M.J. Levine, L.A. Tabak, and M.S. Reddy. 1982. Purification of a low-molecular-weight, mucin-type glycoprotein from human submandibular-sublingual saliva. *Carbohydrate Res.*, 108: 111-122.

Qureshi J.V. and R.J. Gibbons. 1981. Differences in the adsorptive behavior of human strains of Actinomyces viscosus and Actinomyces naeslundii to saliva-treated hydroxyapatite surfaces. *Infect. Immun.*, 131:261-266.

Reinholdt, J. and M. Kilian. 1984. Association between IgA and salivary mucin: nature and site of binding. *J. Dent. Res.*, 63: Special issue. Abstract no. 513.

Revillard, J.P. 1979. β_2 -microglobulin. In: *Phadedoc diagnostic communications*. Ed: A. Annersten. 6: pp 3-23.

Rölla, G. 1980. On the chemistry of the matrix of dental plaque. In: *Microbial adhesion to surfaces*. Eds. Berkeley, R.C.W., I.M. Lynch, J. Melling. P.R. Rutter, and B. Vincent. *Soc. Chem. Indust. Chichester, England, Ellis Horwood Ltd.* pp 425-439.

Rölla, G., J.E. Ciardi, and W.H. Bowen. 1983a. Identification of IgA, IgG, lysozyme, albumin, α -amylase, and glucosyltransferase in the protein layer absorbed to hydroxyapatite from whole saliva. *Scand. J. Dent. Res.*, 91:186-190.

Rölla, G., J.E. Ciardi, and S.A. Schultz. 1983b. Adsorption of glucosyltransferase to saliva coated hydroxyapatite. *Scand. J. Dent. Res.*, 91:112-117.

Rosenberg, M., H. Judes, and E. Weiss. 1983b. Cell surface hydrophobicity of dental plaque microorganisms in situ. *Infect. Immun.*, 42: 831-834.

Rosenberg, M., E. Rosenberg, H. Judes, and E. Weiss. 1983a. Bacterial adherence to hydrocarbons and to surfaces in the oral cavity. *FEMS Microbiol. Let.*, 20:1-5.

Ruoslahti, E. 1981. Fibronectin. *J. Oral Pathol.*, 10:3-13.

Rundegren, J. and Th. Ericson. 1981a. An evaluation of the specificity of salivary agglutinins. *J. Oral Pathol.*, 10: 261-268.

- Rundegren, J., and Th. Ericson. 1981b. Effect of calcium on reactions between a salivary agglutinin and a serotype c strain of Streptococcus mutans. J. Oral Pathol., 10:269-275.
- Salton, M.R.J. 1961. The anatomy of the bacterial surface. Bacteriol. Rev., 25:77-99.
- Schönbeck, C., L. Björck, and G. Kronvall. 1981. Receptor for fibrinogen and aggregated β_2 -microglobulin detected in strains of group B streptococci. Infect. Immun., 31:856-861.
- Sharon, N. and H. Lis. 1975. Use of lectins for the study of membranes. Meth. Membr. Biol., 3:147-200.
- Shomers, J.P., L.A. Tabak, M.J. Levine, I.D. Mandel, and S.A. Ellison. 1982. The isolation of a family of cystein-containing phosphoproteins from human submandibular-sublingual saliva. J. Dent. Res., 61:973-977.
- Simpson, W.A. and E.H. Beachey. 1983. Adherence of group A streptococci to fibronectin on oral epithelial cells. Infect. Immun., 39:275-279.
- Simpson, W.A., D.L. Hasty, J.M. Mason, and E.H. Beachey. 1982. Fibronectin-mediated binding of group A streptococci to human polymorphonuclear leukocytes. Infect. Immun., 37: 805-810.
- Slomiany, B.L., A. Slomiany, and I.D. Mandel. 1980. Lipid composition of human submandibular gland secretion from light and heavy calculus formers. Archs. Oral Biol., 25:749-751.
- Slomiany, A., B.L. Slomiany, and I.D. Mandel. 1981. Lipid composition of human parotid saliva from light and heavy dental calculus-formers. Archs. Oral Biol., 26:151-152.
- Slomiany, A., B.L. Slomiany, Z. Jozwiak, A. Takagi, Y.H. Liau, and I.D. Mandel. 1984a. Identification of fatty acids covalently bound to protein in human submandibular saliva. J. Dent. Res., 63: Special Issue. Abstract No. 903.
- Slomiany, B.L., E. Zdebska, V.L.N. Murty, A. Slomiany, and I.D. Mandel. 1984b. Lipid composition of human pellicle. J. Dent. Res., 63: Special Issue. Abstract No. 905.
- Skurk, A., S. Krebs, and J. Rehberg. 1979. Flow rate, protein, amylase, lysozyme and kallikrein of human parotid saliva in health and disease. Archs. Oral Biol., 24:739-743.
- Slots, J. and R.J. Gibbons. 1978. Attachment of Bacteroides melanogenicus subsp. asaccharolyticus to oral surfaces and its possible role in colonization of the mouth and of periodontal pockets. Infect. Immun., 19:254-264.
- Solheim, B.G. and E. Thorsby. 1974. β_2 -microglobulin is part of the HL-A molecule in the lymphocyte membrane. Nature, 249:36-38.
- Staat, R.H. and J.C. Peyton. 1984. Adherence of oral streptococci: evidence for nonspecific adsorption to saliva-coated hydroxyapatite surfaces. Infect. Immun., 44:653-659.

Svanberg, M., G. Westergren, and J. Olsson. 1984. Oral implantation in humans of Streptococcus mutans strains with different degrees of hydrophobicity. Infect. Immun., 43:817-821.

Sönju, T., A. Aa. Scheie, and K. Pearton. 1984. Bacterial agglutinating potential of some pellicle and salivary proteins. In: Bacterial adhesion and preventive dentistry. Eds: ten Cate J.M., S.A. Leach and J. Arends. IRL Press Ltd (Oxford), pp 51-61.

Tabak, L.A., M.J. Levine, I.D. Mandel, and S.A. Ellison. 1982. Role of salivary mucins in the protection of the oral cavity. J. Oral Pathol., 11:1-17.

Talal, N., H.M. Grey, N. Zvaifler, J.P. Michalski, and T.E. Daniels. 1975. Elevated salivary and synovial fluid β_2 -microglobulin in Sjögren's syndrome and rheumatoid arthritis. Science, 188:1196-1198.

Tandler, B. and J.H. Poulsen. 1976. Fusion of the envelope of mucous droplets with the luminal plasma membrane in acinar cells of the cat submandibular gland. J. Cell. Biol., 68:775-781.

Tatevossian, A. 1981. Kinetics of calcium-binding and release in human dental plaque. In: Tooth surface interactions and preventive dentistry. Eds: Rølla, G., T. Sönju, and G. Embery. IRL Press (London), pp 105-112.

Tomasi, Jr., T.B., and S. Ziegelbaum. 1963. The selective occurrence of γ_1 A globulins in certain body fluids. J. Clin. Invest., 42:1552-1560.

Torpier, G., A. Capron, and M.A. Ouaisi. 1979. Receptor for IgG (Fc) and human β_2 -microglobulin on S. mansoni schistosomula. Nature, 278:447-449.

Underdown, B.J. and K.J. Dorrington. 1974. Studies on the structural and conformational basis for the relative resistance of serum and secretory immunoglobulin A to proteolysis. J. Immunol., 112:949-959.

Williams, R.C. and R.J. Gibbons. 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science, 177:697-699.

Williams, R.C. and R.J. Gibbons. 1975. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun., 11:711-718.

APPENDIX

I

II

III

IV

V

Interactions Between Human Serum Proteins and Oral Streptococci Reveal Occurrence of Receptors for Aggregated β_2 -Microglobulin

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A total of 31 strains of oral streptococci representing *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitior*, *Streptococcus salivarius*, and *Streptococcus milleri* were tested for possible binding of human immunoglobulins G, G1, G2, G3, G4, A1, A2, M1, and M2 and haptoglobin, hemoglobin, fibrinogen, and aggregated β_2 -microglobulin. Radiolabeled β_2 -microglobulin in aggregated form showed affinity for 20 of the 31 strains tested. Binding activity for the protein was found in strains belonging to all five species. The bacterial receptor was resistant to trypsin. Monomeric, unlabeled β_2 -microglobulin did not interfere with the binding of the aggregated form. Of the other proteins tested, only the immunoglobulin A1 protein showed positive binding, and that was only with a single strain of *S. milleri*. β_2 -Microglobulin is present on all nucleated cell membranes in vivo. The reaction between aggregated β_2 -microglobulin and oral streptococci is a new type of human-bacterium interaction which should be considered in studies of bacterial adherence.

Bacteria in the oral cavity are surrounded by a fluid composed of both glandular saliva and gingival exudate. The fluid contains several substances with potential ability to coat or to react with bacterial surface structures. Such reactions may result in a changed ability of the microorganisms to colonize epithelial or tooth surfaces. Thus, Brandtzaeg (5) showed that oral bacteria may be coated with immunoglobulin A (IgA) antibodies. Williams and Gibbons (33) reported that bacteria showed a reduced affinity for epithelial cells after reaction with salivary IgA. Similar effects have been reported for salivary agglutinins (34). It has also been suggested that agglutinins may affect dental plaque formation (22).

During the past few years, it has been shown that antibodies can react not only with their variable, Fab portions with bacterial surface substances but also with their constant, Fc parts. This phenomenon was first shown between protein A from *Staphylococcus aureus* and human immunoglobulins (13), with binding of subclasses IgG1, IgG2, and IgG4 (20) and also IgA2 and IgM2 (15). A similar immunoglobulin binding has also been found among group A, C, and

G streptococci (18). The specificity of group A streptococci is different from both that of group C and G strains and that of protein A (24). An affinity to bacteria has been observed for other human proteins as well, such as fibrinogen (16, 17, 23, 32), β_2 -microglobulin (19), and haptoglobin (29). Such diverse, nonimmune forms of biological affinities have been described as "short-circuit" reactions (G. Kronvall, L. Björck, E. Myhre, and L. Wannamaker, *Proceedings of the 7th International Symposium on Streptococci and Streptococcal Diseases*, in press).

We cannot exclude the possibility that antibodies and other protein substances with ability to short-circuit reactions can be of importance for bacterial colonization. This is particularly true for dental plaque formation, since it is known that dextran production by itself does not give adherence to tooth surfaces (11). To obtain a base for further discussions, we have studied to what extent a number of oral bacteria react with certain human proteins and immunoglobulins which are found in the oral fluid.

MATERIALS AND METHODS

Bacterial strains. The following 31 streptococcal strains were selected for the studies: *Streptococcus mutans* strains AHT and 3720 (serotype a), Fa-1 and

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BHT (serotype b); KPSK2 and NCTC 10449 (serotype c), B 13 and OMZ 176 (serotype d), LM7 (serotype e), OMZ 175 (serotype f), and OMZ 65 (serotype g); *S. sanguis* group 1:A (8) strains OPA1, HPA1, and MPD1, and group 1:B strains KPB1, MPC1, and ATCC 10556; *S. mitior* strains ATCC 9811, NT63, NV71, HV81, and MT61; *S. salivarius* strains NCTC 8618, ATCC 9759, HTS1, MPS1, and MSS2; and *S. milleri* strains NCTC 10708, ATCC 9895, MCP1, and MCP9. With the exception of *S. milleri*, all strains were from the culture collection at Odontologiska Kliniken, University of Göteborg, Göteborg, Sweden. Most of them represent oral isolates, and their origins have been described earlier (6, 9). *S. milleri* strains were kindly provided by S. Edwardsson, University of Lund, Malmö, Sweden. Tubes containing 10 ml of Todd-Hewitt broth were inoculated with each bacterium obtained from fresh blood agar plates. After anaerobic incubation overnight, the bacteria were washed twice in phosphate-buffered saline (0.12 M NaCl, 0.03 M phosphate, pH 7.2) (PBS) containing 0.02% sodium azide (PBSA) and resuspended in PBSA to give a concentration of about 10^9 bacteria per ml. For assays with IgG2, IgG3, and IgG4 (see below), the bacteria were cultivated in a dialyzed yeast extract medium (10).

Protein preparations. The following proteins and immunoglobulins were used: commercially available normal human IgG (Kabi AB, Stockholm, Sweden), fibrinogen (Kabi), haptoglobin (Behringwerke, Marburg, West Germany), and hemoglobin (Sigma, Chemical Co., St. Louis, Mo.). Immunoglobulin preparations from myeloma sera were obtained by preparative agarose electrophoresis (21). The immunoglobulin preparations were diluted in diemal buffer (pH 8.6, containing 0.075 M Ca^{2+}) to a concentration of about 3 mg of protein per ml and then further diluted to a concentration of 1 mg/ml in 0.2 M PBS (pH 7.5) after protein determination at 280 nm. Human β_2 -microglobulin was isolated from urine samples as previously described (4).

^{125}I -labeling. The method of Rosa et al. (30), as modified by Harboe and Folling (15), was used for trace labeling with ^{125}I . Samples (200 to 300 μl) of the protein solutions described above were labeled electrolytically with 0.20 mCi of ^{125}I in a platinum cup for 10 min at 10 μA in an ice-chilled aluminum block. To each iodinated solution were then added 50- μl amounts of 0.05 M NaI and 0.2% human serum albumin (HSA) (Kabi) solutions. Protein-bound ^{125}I was separated from free ^{125}I by dialysis or by gel filtration. After dilution with 2 ml of PBSA containing 0.2% HSA, the protein preparations were dialyzed in a cold room for 18 h against 2 liters of PBSA, which was changed once to 2 liters of fresh PBSA. A Sephadex PD-10 (Pharmacia) column equilibrated with 0.2% HSA in PBSA was used in some cases.

After separation of free ^{125}I , the protein preparations were diluted in PBSA containing 0.2% HSA to give a radioactivity of 5,000 to 15,000 cpm/25 μl as measured in an LKB Wallac 1270 Rackgamma counter (Biotec AB, Stockholm, Sweden).

Aggregated β_2 -microglobulin. Purified β_2 -microglobulin was suspended in 0.2 M PBS (pH 7.5), and 200 μl (200 μg) was electrolytically labeled with 0.5

mCi of ^{125}I . Preparations of aggregated β_2 -microglobulin were then obtained by using glutaric dialdehyde as a coupling reagent by the method of Avrameas (2). The radiolabeled aggregated β_2 -microglobulin was gel filtered on a Sephadex G-200 column equilibrated with PBSA containing 0.05% Tween 20. Fractions of β_2 -microglobulin with a molecular weight of approximately 275,000 were eluted from the column and suspended in PBSA containing 0.2% HSA.

Binding assay. Binding of ^{125}I -labeled protein to streptococci was investigated by mixing a 25- μl sample of labeled protein with 200 μl of bacterial suspension (2×10^8 bacteria). The mixture was incubated for 1 h at room temperature. After washing with 2 ml of PBSA containing 0.05% Tween 20, the suspension was centrifuged for 10 min at $2,000 \times g$, and the radioactivity in the pellet was measured. The uptake of ^{125}I -labeled protein in the pellet was compared with the total radioactivity in 25 μl of labeled protein solution and expressed as a percentage of this value.

Inhibition studies. Unlabeled monomeric β_2 -microglobulin was added before uptake assays for studies of possible inhibition. A 50- μl (100- μg) sample of the monomeric protein solution was added to 200- μl *S. sanguis* KPB1, *S. salivarius* 9759, *S. mitior* NV71, and *S. milleri* MCP1 suspensions. After 1 h at room temperature, radiolabeled aggregated β_2 -microglobulin was added, and binding assays were performed as described above.

Trypsin digestion. Suspensions of *S. mutans* B13, *S. sanguis* MPC1, *S. salivarius* 9759, and *S. mitior* NV71 were mixed with equal volumes of the β_2 -microglobulin-negative strain *Staphylococcus albus* L 603. The mixture was done to reduce the number of test bacteria, thus giving a more sensitive system for binding studies but still with a pellet of convenient size. *S. milleri* MCP1 suspensions were used nondiluted. Varying amounts of trypsin (Sigma) were added to 1-ml samples of the suspensions, and the mixtures were incubated at 37°C. After 20 min the digestion was stopped by the addition of Trasylol (Bayer) and subsequent washings. The bacteria were then resuspended in PBSA and tested for uptake of aggregated β_2 -microglobulin. Two trypsin-sensitive strains of Lancefield group A streptococci served as positive controls.

RESULTS

Immunoglobulin preparations, fibrinogen, haptoglobin, and hemoglobin in binding studies. Twelve different human proteins, polyclonal IgG, monoclonal IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, fibrinogen, haptoglobin, and hemoglobin, were radiolabeled and tested for binding to 31 streptococcal strains (Table 1). No positive reactions were demonstrated for any of these strains with trace-labeled proteins with one single exception. These negative results are in contrast to the marked binding activities previously noted for group A, C, and G streptococcal strains (18, 24). The one exception was a 19% uptake of the IgA1 myeloma protein to one strain of *S. milleri* (MCP9).

TABLE 1. Binding of human serum proteins to oral streptococci^a

Organism	No. of strains tested	Uptake of:											
		IgG	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM1	IgM2	Aggregated β_2 -microglobulin	Fibrinogen	Haptoglobin
<i>S. mutans</i> serotypes a through g	11	—	—	—	—	—	—	—	—	—	8	—	—
<i>S. sanguis</i> groups I: A and I:B	6	—	—	—	—	—	—	—	—	—	3	—	—
<i>S. salivarius</i>	5	—	—	—	—	—	—	—	—	—	3	—	—
<i>S. mitior</i>	5	—	—	—	—	—	—	—	—	—	3	—	—
<i>S. milleri</i>	4	—	—	—	—	—	1	—	—	—	3	—	—

^a A total of 31 strains were tested for uptake of 13 different, radiolabeled human proteins. Only aggregated β_2 -microglobulin and IgA showed binding, with the number of positive strains indicated in the table. —, Negative reactions with all strains.

Uptake of human radiolabeled aggregated β_2 -microglobulin. Of 31 oral streptococcal strains tested, 20 showed an uptake of aggregated β_2 -microglobulin of more than 10% of the added protein (Fig. 1 and Table 1). The reactivity was detected with all five bacterial species studied. Of the *S. mutans* strains tested, strains of serotypes a, c, d, f, and g showed an uptake of the protein. Among the *S. sanguis* strains, only the group I:B strains were reactive. The other species tested showed both positive and negative strains. A 10% level for significant binding gave a clear-cut separation of positive and negative strains in previous studies and represents a definite affinity between the protein and the strep-

tococcal strains (19). In the present study there was no definite clustering of binding levels among the strains (Fig. 1). The uptake values presented in Fig. 1 represent mean values of repeated experiments. The uptake variations for the highly reactive strains were within $\pm 10\%$ and for the negative strains were within $\pm 2\%$.

Effect of added unlabeled monomeric β_2 -microglobulin. Inhibition experiments were performed to study the effect of added unlabeled, monomeric β_2 -microglobulin in the assay. The addition of monomeric, non-radiolabeled β_2 -microglobulin did not affect the uptake of the aggregated, radiolabeled form of the protein.

Effect of trypsin digestion. Trypsin digestion experiments were performed to study the enzyme resistance of the β_2 -microglobulin receptor of oral streptococci. There was no significant effect of trypsin digestion of five different streptococcal strains on their uptake of aggregated β_2 -microglobulin, not even at high concentrations of trypsin. Additional experiments using up to 400 μg of trypsin added to 1 ml of suspension confirmed the relative trypsin resistance of the receptor.

DISCUSSION

Complex interactions between bacteria and the host seem to govern the outcome of a possible adherence (11). Various nonimmune interactions, termed short circuits because of their seemingly irrational crossing of normal biological pathways (Kronvall et al., in press), might interfere with adherence mechanisms. Such interference has been clearly demonstrated between normal IgA antibodies and oral streptococci (33). A few studies have also shown that salivary IgA antibodies can be found reacting with oral bacteria of serotypes not commonly

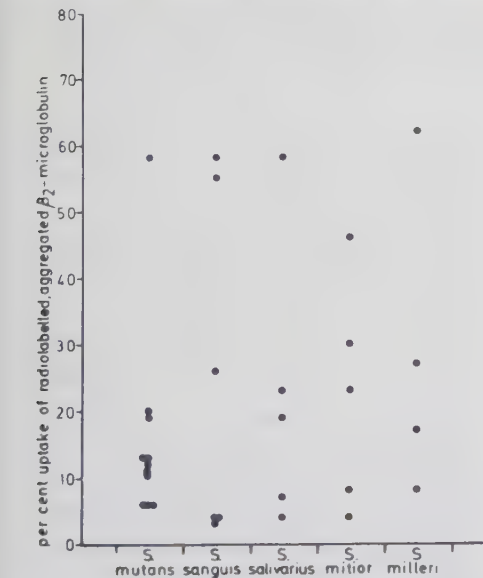


FIG. 1. Uptake of radiolabeled, aggregated β_2 -microglobulin by oral streptococci.

found in the populations examined (1, 7). Binding mediated by the Fc portions of the antibodies are among the possibilities suggested to explain this fact. Although data for the secretory component or for IgA combined with this component are not yet available, the results of the present study indicate that nonimmune protein binding apparently is not very common among oral streptococci (Table 1).

For the 12 human proteins studied other than β_2 -microglobulin, there was no consistent binding reactivity noted with oral streptococci. These findings are in contrast to other results concerning binding to β -hemolytic streptococci belonging to Lancefield groups A, C, and G (Kronvall et al., in press). Such strains were highly reactive with several human proteins: IgG, IgA, to a lesser extent IgM, fibrinogen, aggregated β_2 -microglobulin, and, in some cases, also haptoglobin. Some role for these short circuits in host-parasite relationships should be looked for. The negative findings among oral streptococci emphasize the marked differences between these strains and group A, C, and G streptococci, for instance regarding antigenic characteristics, metabolic capacities, and pathogenicity.

Of the 13 human proteins tested, 1 showed a definite reactivity with oral streptococci: aggregated β_2 -microglobulin bound significantly to 20 of the 31 strains tested (Table 1 and Fig. 1). Such a reactivity has been found previously among group A, C, and G streptococci (19). In the *S. mutans* and *S. sanguis* strains, there may be a correlation between protein binding and serotype, but more strains are needed for definite conclusions. Additional tests were performed to further study the binding structures found on oral streptococci. These experiments included inhibition tests with unlabeled monomeric β_2 -microglobulin and also trypsin digestion tests. Addition of β_2 -microglobulin in the monomeric form did not inhibit the uptake of the aggregated protein. Similar results have been obtained for the β_2 -microglobulin receptor on Lancefield group A, C, and G streptococci.

Trypsin digestion of five different strains showing affinity to aggregated β_2 -microglobulin did not seem to affect the uptake of aggregated β_2 -microglobulin, not even at high concentrations of trypsin (400 μ g). These results were rather unexpected, as streptococci belonging to Lancefield groups A, C, and G, showing high reactivity with this protein, were highly sensitive to trypsin digestion (19). The differences in trypsin sensitivity, with extreme sensitivity in all group A, C, and G streptococci tested compared with a relatively marked resistance for all five oral streptococci, are in line with other major

differences between these two groups of organisms. This does not necessarily imply that the basic receptor structures for β_2 -microglobulin are different. The requirement of an aggregated form of β_2 -microglobulin in both cases would, in fact, support a common basic type of receptor. In such a case the evolutionary origin of these sequences must be very old. Differences in trypsin resistance might indicate that the structural incorporation of the receptor is different in the two groups. This might be of interest concerning the possible localization of the receptor for M-protein in A, C, and G streptococci (Kronvall et al., in press).

Human β_2 -microglobulin was originally found in free form in various body fluids (4). It is a protein with a molecular weight of only 11,800. Recent work has shown that β_2 -microglobulin is structurally related to the immunoglobulins (12, 26, 31). Findings summarized by Berggård (3) indicate that in saliva and other body fluids β_2 -microglobulin may appear in a soluble monomeric form. The protein is also a major cell membrane protein (26, 28) and occurs linked to the major histocompatibility antigens (14, 25, 27). It is thus present on the epithelial cells of the oral cavity to which the oral streptococci normally adhere. The presence of receptors for aggregated β_2 -microglobulin on several strains of streptococci might therefore influence the capacity of these strains to adhere to nucleated cells.

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LITERATURE CITED

1. Arnold, R. R., J. Mestecky, and J. R. McGhee. 1976. Naturally occurring secretory immunoglobulin A antibodies to *Streptococcus mutans* in human colostrum and saliva. *Infect. Immun.* 14:355-362.
2. Avrameas, S. 1969. Coupling of enzymes to protein with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* 6:43-52.
3. Berggård, I. 1976. β_2 -Microglobulins: isolation, properties and distribution. *Fed. Proc.* 35:1167-1170.
4. Berggård, I., and A. G. Bearn. 1968. Isolation and properties of a low molecular weight β_2 -microglobulin occurring in human biological fluids. *J. Biol. Chem.* 243:4095-4103.
5. Brandtzaeg, P., I. Fjellanger, and S. T. Gjeruldsen. 1968. Adsorption of immunoglobulin A onto oral bacteria in vivo. *J. Bacteriol.* 96:242-249.
6. Bratthall, D. 1972. Immunofluorescent identification of *Streptococcus mutans*. *Odontol. Revy* 23:181-196.
7. Bratthall, D., L. Gahnberg, and B. Kræse. 1978. A method for detection of IgA antibodies to *Streptococcus mutans* serotypes in parotid saliva. *Arch. Oral Biol.* 23:843-849.
8. Carlsson, J. 1967. Presence of various types of non-haemolytic streptococci in dental plaque and in other sites

- of the oral cavity in man. *Odontol. Revy* 18:55-74.
9. Carlsson, J. 1968. A numerical taxonomic study of human oral streptococci. *Odontol. Revy* 19:137-160.
 10. Carlsson, J., E. Newbrun, and B. Krasse. 1969. Purification and properties of dextranucrase from *Streptococcus sanguis*. *Arch. Oral Biol.* 14:469-478.
 11. Clark, W. B., L. L. Bammann, and R. J. Gibbons. 1978. Ability of *Streptococcus mutans* and a glycosyltransferase-defective mutant to colonize rodents and attach to hydroxylapatite surfaces. *Infect. Immun.* 21:681-684.
 12. Cunningham, B. A., J. L. Wang, I. Berggård, and P. A. Peterson. 1973. The complete amino acid sequence of β_2 -microglobulin. *Biochemistry* 12:4811-4822.
 13. Forsgren, A., and J. Sjoquist. 1966. Protein A from *S. aureus*. I. Pseudoimmune reaction with human γ -globulin. *J. Immunol.* 97:822-827.
 14. Grey, H. M., R. T. Kubo, S. M. Colon, M. D. Poulik, P. Cresswell, T. Springer, M. Turner, and J. L. Strominger. 1973. The small subunit of HL-A antigens is β_2 -microglobulin. *J. Exp. Med.* 138:1608-1612.
 15. Harboe, M., and I. Folling. 1974. Recognition of two distinct groups of human IgM and IgA based on different binding to staphylococci. *Scand. J. Immunol.* 3:471-482.
 16. Hawinger, J., D. K. Hammond, and S. Timmons. 1975. Human fibrinogen possesses binding site for staphylococci on A α and B β polypeptide chains. *Nature (London)* 258:643-645.
 17. Kantor, F. S. 1965. Fibrinogen precipitation by streptococcal M-protein. I. Identity of the reactants and stoichiometry of the reaction. *J. Exp. Med.* 121:849-859.
 18. Kronvall, G. 1973. A surface component in group A, C and G streptococci with non-immune reactivity for immunoglobulin G. *J. Immunol.* 111:1401-1406.
 19. Kronvall, G., E. B. Myhre, L. Björck, and I. Berggård. 1978. Binding of aggregated β_2 -microglobulin to surface protein structure in groups A, C and G streptococci. *Infect. Immun.* 22:136-142.
 20. Kronvall, G., and R. C. Williams, Jr. 1969. Differences in antiprotein A activity among IgG subgroups. *J. Immunol.* 103:828-833.
 21. Laurell, C.-B. 1965. Antigen-antibody crossed electrophoresis. *Anal. Biochem.* 10:358-361.
 22. Magnusson, I., and T. Ericson. 1976. Effect of salivary agglutinins on reactions between hydroxylapatite and a serotype c strain of *Streptococcus mutans*. *Caries Res.* 10:273-286.
 23. Much, H. 1908. Über eine Vorstufe des Fibrinfermentes in Kulturen von *Staphylokokkus aureus*. *Biochem. Z.* 14:143-155.
 24. Myhre, E. B., and G. Kronvall. 1977. Heterogeneity of nonimmune immunoglobulin Fc reactivity among gram-positive cocci: description of three major types of receptors for human immunoglobulin G. *Infect. Immun.* 17:475-482.
 25. Nakamuro, K., N. Tanigaki, and D. Pressman. 1973. Multiple common properties of human β_2 -microglobulin and the common portion fragment derived from HL-A antigen molecules. *Proc. Natl. Acad. Sci. U.S.A.* 70:2863-2865.
 26. Peterson, P. A., B. A. Cunningham, I. Berggård, and G. M. Edelman. 1972. β_2 -Microglobulin—a free immunoglobulin domain. *Proc. Natl. Acad. Sci. U.S.A.* 69:1697-1701.
 27. Peterson, P. A., L. Rask, and J. B. Lindblom. 1974. Highly purified papainsolubilized HL-A antigens contain β_2 -microglobulin. *Proc. Natl. Acad. Sci. U.S.A.* 71:35-39.
 28. Poulik, M. D. 1973. Presence of β_2 -microglobulin on B- and T-cells and lymphocytotoxicity of anti- β_2 -microglobulin sera. *Immunol. Commun.* 2:403-414.
 29. Prokop, O., W. Kohler, and G. Gesorick. 1977. Untersuchungen zu den Beziehungen zwischen Haptoglobintyp und Streptokokken der Gruppe G. (Relationship between haptoglobin types and group G streptococci). *Z. Immunitätsforsch.* 153:457-465.
 30. Rosa, U., G. A. Scassellati, F. Pennisi, N. Riccioni, P. Giagnoni, and R. Giordani. 1964. Labelling of human fibrinogen with ^{131}I by electrolytic iodination. *Biochim. Biophys. Acta* 86:519-526.
 31. Smithies, O., and M. D. Poulik. 1972. Initiation of protein synthesis at an unusual position in an immunoglobulin gene. *Science* 175:187-189.
 32. Tillett, W. S., and R. L. Garner. 1934. The agglutination of hemolytic streptococci by plasma and fibrinogen. A comparison of the phenomenon to serological relations with the same organisms. *Bull. Johns Hopkins Hosp.* 54:145-156.
 33. Williams, R. C., and R. J. Gibbons. 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science* 177:697-699.
 34. Williams, R. C., and R. J. Gibbons. 1975. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. *Infect. Immun.* 11:711-718.

Further Characteristics of β_2 -Microglobulin Binding to Oral Streptococci

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A total of 85 strains of oral bacteria representing *Streptococcus mutans*, *S. sanguis*, *S. mitior*, *S. salivarius*, *S. milleri*, *S. infrequens*, *S. durans*, *S. lactis*, *S. faecalis*, *S. faecium*, *S. equinus*, *Streptococcus* species group E, *Actinomyces*, and one group A *Streptococcus* were tested for binding of aggregated human β_2 -microglobulin. Positive affinity between bacteria and aggregated human β_2 -microglobulin was detected in 36% of the strains. No apparent correlation with bacterial species, serotype, or group was noted. No positive strains were detected among seven group I:A *S. sanguis* strains ($P < 0.01$). Binding constants for one *S. mutans* strain indicated heterogeneous binding structures on the bacterial surface. The number of binding sites for aggregates of human β_2 -microglobulin involving multipoint attachment varied from 70 to 1,700 per bacterial cell. With whole saliva as buffer, a general increase in affinity was seen. Variations in salt concentrations of the buffers revealed different salt-dependent species-associated uptake patterns. Oral bacteria tended to have an uptake maximum at a salt concentration similar to that seen in saliva. Binding structures for aggregated β_2 -microglobulin on oral streptococci were sensitive to pepsin, heat, and formaldehyde treatment. Bacterial binding structures for aggregated β_2 -microglobulin might represent one of several factors of importance for bacterial attachment in the oral cavity. Experimental conditions reflecting the salivary milieu increased the degree of interaction, emphasizing the importance of physiological test systems for such studies.

Surfaces of the oral cavity are colonized by large numbers of bacteria of various species. A common and fundamental characteristic of these bacteria is their capacity to attach to host surface structures (10). Different mechanisms responsible for bacterial attachment in the mouth have been increasingly studied during the past few years. Considerable interest has been directed toward the effects of saliva on bacterial attachment. Secretory immunoglobulin A in saliva is, for example, capable of inhibiting the attachment of *S. sanguis* to oral epithelial cells (25). High-molecular-weight glycoproteins in saliva, termed agglutinins, are capable of aggregating bacteria (12) and could thereby possibly prevent bacterial attachment (26). The agglutinins may also be absorbed onto teeth in pellicle formation, providing receptors for oral bacteria and perhaps facilitating the formation of dental plaque (18, 19). Other substances may interact with bacteria or host surfaces and affect the ability of bacteria to colonize the surfaces of the oral cavity (11, 17).

Relatively little is known about host surface substances involved in bacterium-host interactions in the oral cavity. β_2 -Microglobulin, the light chain of HL-A antigens, is present on oral

epithelial cell surfaces and in saliva (for references, see reference 2) and might therefore be worth considering when approaching this problem. Interactions between several strains of oral streptococci and aggregated β_2 -microglobulin have been described earlier (8). In the present investigation, we studied the occurrence of such receptors as well as the kinetics of the interactions and the physicochemical characteristics of the receptor structures on several species of oral bacteria with affinity for aggregated β_2 -microglobulin.

MATERIALS AND METHODS

Bacterial strains. A total of 85 strains of oral bacteria were selected for study: 22 strains of *Streptococcus mutans* (AHT and 3720 [serotype a], Fa-1 and BHT [serotype b], KPSK2, NCTC 10449, PK1, B21, P8, S8, At3, At6, and OMZ70 [serotype c], B13, OMZ176, OIHI, ME1, and MX [serotype d], LM7 and P4 [serotype e], OMZ175 [serotype f], and OMZ65 [serotype g]); 22 strains of *S. sanguis* (OPA1, HPA1, MPD1, MPG1, LPA1, KTD1, and MPB1 [group I:A] [5], KPB1, MPC1, ATCC 10556, 804, 903, MPH1, KPE2, PPE21, OPC1, OSH1, HPC1, PPF1, and GVE1 [group I:B] [5], G26-R, and G26-S [24]); 11 strains of *S. mitior* (ATCC 9811, Nt62, LV61, and KPD1 [group V:A] [5], Nt63, Nt61, Mt61, and Pt51 [group V:B]

[5], Nv71 and Hv81 [group IV] [5] and CHT [15]); 11 strains of *S. salivarius* (PVS2, GSS2, MVS1, KTS1, KPS1, NCTC 8618, ATCC 9759, HTS1, MPS1, MSS2, and HHT [group III] [5] [15]); and 5 strains of *S. milleri* (NCTC 10708, ATCC 9895, MCPI, MCP9, and 1624 [freshly isolated from a brain abscess]). Seven other *Streptococcus* species were also used: Lancefield's group E strains NCTC 5385, NCTC 5968, 012, 073, 074, 0232, and 0239. In addition, we studied *S. infrequens* strain NCTC 4678, *Streptococcus durans* strain NCDO 596, *S. lactis* strain NCTC 6681, *S. faecalis* strain NCTC 775, *S. faecium* strain NCTC 7171, *S. equinus* strain ATCC 9812, and *Actinomyces* strain T6. The strains were selected from the culture collection of the Department of Cariology, University of Lund, Malmö, and from a fresh isolate from the Department of Medical Microbiology, University of Lund, Lund, Sweden. The origins of the strains have been described earlier (4-6, 15, 24).

Bacterial strains were kept on blood agar plates and transferred to tubes containing 10 ml of Todd-Hewitt broth. After incubation in anaerobic jars overnight the bacteria were washed twice in phosphate-buffered saline (0.12 M NaCl-0.03 M phosphate, pH 7.2; PBS) containing 0.02% NaN₃ and 0.05% Tween 20 (PBSAT) and resuspended in the same buffer to a concentration of 10¹⁰ bacteria per ml as determined by measuring the optical density at 530 nm in a Beckman colorimeter model CP-1. A standard curve was obtained through direct counting of bacterial cells in a Petroff-Hausser chamber. In some experiments, alternative buffers were used as indicated.

Protein preparations. Human β_2 -microglobulin and α_1 -microglobulin were purified as previously described (3, 7). Proteins were radiolabeled with ¹²⁵I by the chloramine-T method (13) and aggregated with glutaric dialdehyde according to Avrameas (1) as earlier described (8, 16). Aggregates with a molecular weight of approximately 280,000 were used as determined from gel filtration on a Sephadex G-200 column.

Binding assays. To 25 μ l (0.3 μ g) of radiolabeled aggregated protein solution, 200 μ l of bacterial suspension was added. After incubation for 1 h at room temperature, the suspension was washed with 2 ml of PBSAT and centrifuged for 10 min at 2,000 $\times$ g, and the radioactivity in the pellet was measured in a LKB-Wallac 1270 Rackgamma gamma counter (AB Biotec, Stockholm, Sweden). The radioactivity in the pellet was expressed as percentage of the total radioactivity added.

Calculation of binding constant and number of receptors. Radiolabeled, aggregated β_2 -microglobulin with an apparent molecular weight of 280,000 was diluted serially in PBSAT. To 100 μ l of the different dilutions of aggregated β_2 -microglobulin, 200- μ l bacterial suspensions of *S. mutans* ME1 were added. The suspensions were incubated, washed, and spun down as described above. Binding constants and the approximate number of binding structures on each bacterium were calculated according to Scatchard (22).

Influence of pH changes in the buffer environment. Buffers with different pH values were prepared by adjusting 0.15 M acetate and 0.15 M primary phosphate containing 0.05% Tween 20 with 5 N NaOH. The uptake of aggregated β_2 -microglobulin to two

strains of oral streptococci was tested at different pH values from 3.0 to 8.0. Then 250- μ l samples of the bacterial suspensions were washed, spun down, and resuspended in the respective buffers. For uptake assays as described above, 200- μ l samples of the bacterial suspensions were assayed using the respective buffers throughout. *Staphylococcus albus* strain L603 (heat killed) and tubes containing no bacteria served as negative controls.

Saliva as buffer. To investigate the possible effects of saliva on the uptake of aggregated β_2 -microglobulin to oral streptococci, 15 different strains were tested in binding assays as described above. Freshly collected paraffin-stimulated whole saliva from five persons was centrifuged to remove debris, pooled, and used as the buffer throughout the assay. From conductivity measurement of saliva samples, a buffer with the same conductivity was prepared. With this 0.066 M PBS buffer containing 0.05% Tween 20 used throughout the assay, 77 strains of bacteria were tested for binding of human aggregated β_2 -microglobulin.

Influence of changes in ionic strength. Series of buffers with different ionic strengths were prepared in order to investigate the effect of different salt concentrations on the binding of aggregated β_2 -microglobulin to oral streptococci as compared with one group A *Streptococcus* strain, AR-1, and *Staphylococcus aureus* Cowan I. To 0.01 M phosphate buffer containing 0.05% Tween 20, various amounts of NaCl were added, giving buffers with ionic strengths from 0.015 to 0.48 M. Twelve strains of bacteria were included in uptake assays as described above, using the respective buffers throughout the assay. Tubes containing no bacteria served as negative controls.

Fibrinogen inhibition studies. To 200- μ l suspensions of four different oral streptococci, various amounts of fibrinogen (AB Kabi, Stockholm, Sweden) were added to study the possible inhibition of bacterial binding of aggregated β_2 -microglobulin. The suspensions were incubated at room temperature for 30 min and washed in 2 ml of PBSAT. Bacteria were resuspended in 200 μ l of PBSAT and used for uptake assays as described above.

Pepsin digestion. Pellets from 250- μ l bacterial suspensions of three strains of oral streptococci were suspended in 0.1 M acetate buffer (pH 4.5) containing various amounts of pepsin (Sigma Chemical Co., St. Louis, Mo.; N:o P-7012, lot. 26C-8045) and incubated in a water bath for 1 h at 37°C. Pepsin digestion was stopped by the addition of 50 μ l of 1 M tris-(hydroxymethyl)aminomethane buffer at room temperature. The bacterial suspensions were spun down, washed in 2 ml of PBSAT, and resuspended in 250 μ l of PBSAT. For uptake assays as described above, 200- μ l samples of the suspensions were used.

Formaldehyde treatment. Washed pellets of 250- μ l bacterial suspensions of two strains of oral streptococci were resuspended in 2 ml of PBSAT containing formaldehyde concentrations of 0 to 0.5%. The suspensions were incubated at room temperature for 30 min, washed, and resuspended in PBSAT. For uptake assays as described above, 200- μ l samples of the suspensions were used.

Heat treatment. The possible heat sensitivity of the bacterial binding structures for aggregated β_2 -mi-

croglobulin was investigated. For this assay, 400- μ l bacterial suspensions of *S. mutans* B13 and ME1 and *S. sanguis* MPC1 were incubated at different temperatures in a water bath for 10 min. For uptake assays as described above, 200- μ l samples of the bacterial suspensions were used.

RESULTS

Binding of aggregated β_2 -microglobulin to oral bacteria. A total of 84 strains of oral streptococci were tested for binding of human aggregated β_2 -microglobulin (Fig. 1). Protein uptake varied from 1.5 to 31% of the added protein, with 36% of the strains showing an uptake of aggregated β_2 -microglobulin of more than 4%. In Fig. 1, a cluster of negative strains is seen around the 2.5% uptake level, clearly separated from the positive strains with an uptake of over 4%. When comparing levels of binding among different groups or serotypes of bacteria, consistent binding was seen within certain serotypes and groups in repeated assays. All strains of *S. mutans* serotypes *d*, *f*, and *g* showed positive binding, whereas all strains of serotypes *b* and *e* were

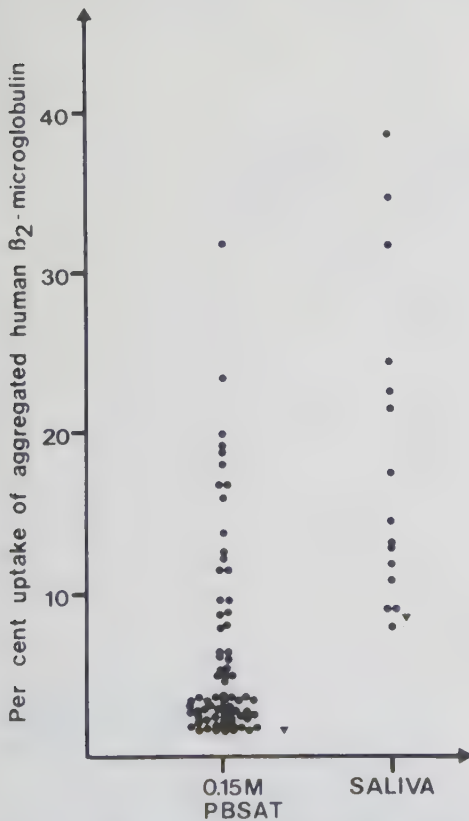


FIG. 1. Uptake of labeled aggregated β_2 -microglobulin to oral streptococci in PBS and in saliva. Symbols: ∇ , empty tube; $\blacktriangledown$, L603.

negative. Both positive and negative strains were seen within serotypes *a* and *c*. *S. mitior* group V:A and IV strains were all negative; in group V:B, one of four strains was positive. Two of 11 *S. salivarius* strains tested were positive, as were 3 of 5 *S. milleri* strains. The one strain of *Actinomyces* showed a positive binding with a 14% uptake. Within *S. sanguis* group I:A, all seven strains were negative in contrast to group I:B, which showed both positive and negative strains. With so few strains of each serotype and group tested, a statistically significant correlation between group and β_2 -microglobulin binding was only seen in a negative direction for *S. sanguis* group I:A ($P < 0.01$). Other species of streptococci tested were nonhomogeneous in binding of the protein.

The monomeric form of human β_2 -microglobulin was studied in control experiments. Radio-labeled monomeric β_2 -microglobulin was tested for affinity to seven strains of oral streptococci, using 0.066 M PBS containing 0.05% Tween 20 as the buffer throughout the assay. Four strains of oral streptococci were also tested for affinity for the protein in a pH 5.6 acetate buffer (0.15 M) containing 0.05% Tween 20. None of the oral streptococci tested showed affinity for the protein, not even at the low pH. Previous studies have shown that monomeric β_2 -microglobulin does not interfere with binding of the aggregated form of the protein (8, 16). Eight strains of oral streptococci, both positive and negative in affinity for aggregated β_2 -microglobulin, were tested for affinity for aggregated human α_1 -microglobulin to investigate the possible effects of aggregation of proteins. In this control experiment, none of the strains showed affinity for aggregated human α_1 -microglobulin.

Determination of binding constant and binding capacity for aggregated human β_2 -microglobulin. *S. mutans* ME1 was tested for binding of varying amounts of aggregated β_2 -microglobulin, using a preparation with an apparent molecular weight of 280,000 achieved by rechromatography on a Sephadex G-200 column. Bound (*b*) and free (*f*) fractions of radio-labeled protein were determined as described by Scatchard (22), using the relationship $b/f = nK - bK$ (Fig. 2). The regression curve in Fig. 2 is divided into two linear regression lines, *a* and *b*, suggesting a heterogeneous receptor on bacteria for the protein. The slope of the regression line *a* ($r^2 = 0.88$) indicates one type of receptor on bacteria with a high affinity but with a very low capacity. The slope of line *b* ($r^2 = 0.78$) indicates a receptor with lower affinity but higher capacity. The interceptions of these lines with the *x*-axis give the value *n*, the amount of aggregated β_2 -microglobulin bound at saturation. Calcula-

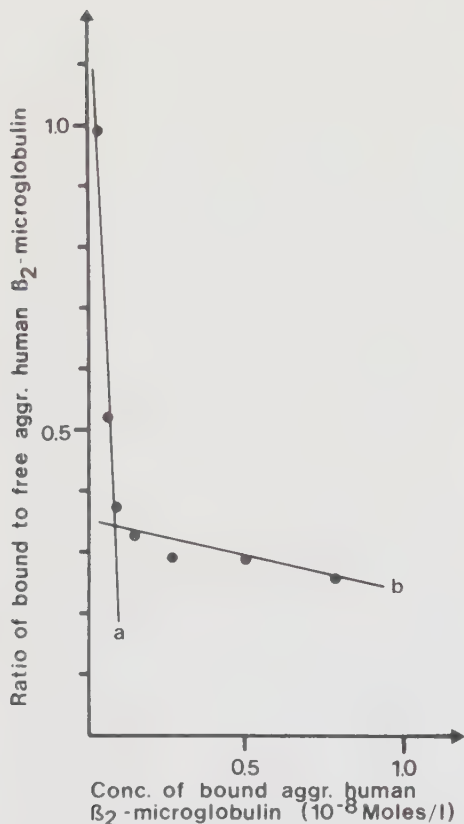


FIG. 2. Scatchard plots of binding experiments performed with varying concentrations of labeled aggregated β_2 -microglobulin. The *S. mutans* strain ME1 was studied.

tion of binding constants for the two receptors according to the formula above, using a molecular weight of 280,000, gives apparent binding constants (K) of 11×10^8 liters/mol for the high-affinity receptor and 0.12×10^8 liters/mol for the low-affinity receptor. The binding capacities were calculated to 0.12×10^{-8} mol/liter for the high-affinity receptor and to 2.9×10^{-8} mol/liter for the low-affinity receptor. This gives a maximum binding capacity of 70 aggregates of β_2 -microglobulin with a mean molecular weight of 280,000 on each bacterium to the high-affinity receptor and of 1,700 aggregates to the low-affinity receptor. Because of the multivalent nature of aggregated β_2 -microglobulin, the calculated constants should be used only for comparative purposes and not taken as absolute figures.

Effect of changes of hydrogen ion concentration on the binding of aggregated β_2 -microglobulin to oral bacteria. Two strains of oral streptococci were suspended in buffers with different pH values and tested for affinity to aggregated human β_2 -microglobulin. In Fig. 3,

the effect of pH changes on the affinity of aggregated β_2 -microglobulin for oral streptococci is presented. Generally, a decrease of pH below pH 6 gave an increase of affinity for both positive and control strains. The β_2 -microglobulin-positive strains of oral streptococci seemed to increase in affinity faster than did the control strain, *Staphylococcus albus* L603, when pH was decreased. With an increase of pH over 6, *S. sanguis* MPC1 showed a slow increase in affinity for the protein. The buffers used in the systems were changed between pH 5.75 and 6.0 from 0.15 M acetate buffer to 0.15 M phosphate buffer, both containing 0.05% Tween 20. At pH 5.5, which represents a hydrogen ion concentration not uncommon in the oral cavity, a clear discrepancy in affinity for aggregated β_2 -microglobulin was seen between the control strain and the positive oral streptococci. At this pH there was also a high background affinity for the plastic tubes.

Effect of saliva as buffer on the uptake of normal human aggregated β_2 -microglobulin. Fifteen strains of oral streptococci were tested for affinity to aggregated β_2 -microglobulin, using saliva as the buffer throughout the

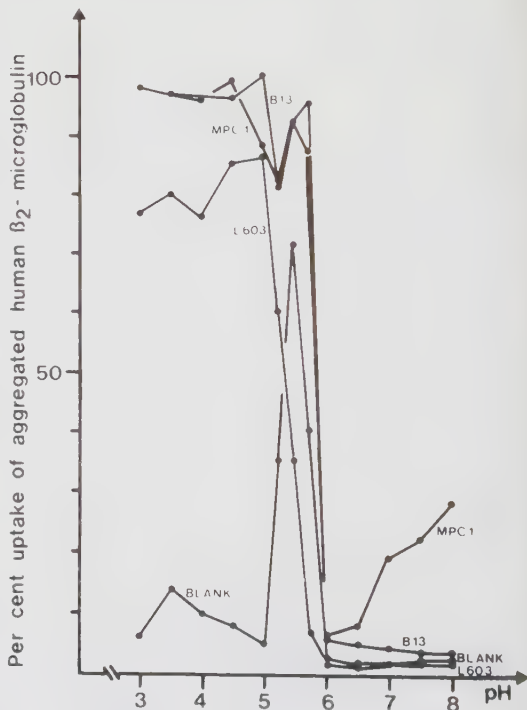


FIG. 3. Effects of pH variation in buffers on the uptake of labeled aggregated β_2 -microglobulin to *Streptococcus mutans* B13 and *Streptococcus sanguis* MPC1. Negative controls were *Staphylococcus albus* L603 and empty tubes.

assay (Fig. 1). A general increase in affinity was seen in this system. As compared with a positive control strain, group A *Streptococcus* strain AR-1, the general increase of affinity between oral streptococci and the protein was 2.2 times the increase of AR-1 binding when compared with the uptake in 0.066 M PBS with 0.05% Tween 20 (see below).

Effect of ionic strength on the uptake of aggregated β_2 -microglobulin. A total of 77 bacterial strains were tested for affinity to aggregated normal human β_2 -microglobulin, using 0.066 M PBS containing 0.05% Tween 20 as the buffer throughout the assay. The salt concentration in this buffer is similar to that in saliva. Compared with the binding of the protein in a 0.15 M PBS buffer with Tween 20 to the same bacteria, a general increase of affinity was noted. A clustering of negative strains was seen around the 2.5% uptake level. Compared with the affinity seen in 0.15 M PBS, the correlations between species, serotypes, or groups were similar with two notable exceptions. All strains of *S. milleri* showed positive binding in 0.066 M PBS, but only three of five strains did so in 0.15 M PBS. Similar effects were seen for the *S. salivarius* strains: only 2 of 11 strains were positive in 0.15 M PBS, whereas 9 of 10 strains became positive in 0.066 M PBS. Twelve strains of bacteria were tested for affinity to aggregated β_2 -microglobulin in buffers with ionic strength ranging from 0.015 to 0.48 M. The oral streptococci had, in general,

a maximum uptake at a lower salt concentration than did the group A *Streptococcus* strain AR-1 (Fig. 4). At least four patterns of salt concentration dependence were seen in Fig. 4: (i) the positive strains *S. sanguis* MPC1 and PPE21 and *S. milleri* MCP9 and 9895 showed an uptake maximum at a salt concentration close to 0.03 M, which decreased when more salt was added in the buffers; (ii) the positive strains *S. mutans* B13 and ME1 showed an uptake maximum close to 0.08 M; (iii) the negative strains *S. mutans* LM7 and *S. sanguis* OPA1 showed an uptake of protein only at 0.015 M, and when salt was added no uptake was seen; and (iv) the β_2 -microglobulin-positive group A *Streptococcus* strain AR-1 showed an uptake maximum at 0.15 M. *S. milleri* strain MCP1 showed an uptake maximum at 0.015 M but did not lose affinity as quickly as negative strains when more salt was added. The two negative strains *Staphylococcus aureus* Cowan I and *S. albus* L603 did not show any uptake of added protein except for a small increase at 0.48 M.

Inhibition studies using fibrinogen pretreatment. Four positive strains of oral streptococci were pretreated with fibrinogen before uptake assays with aggregated normal human β_2 -microglobulin to determine whether the receptor on oral streptococci could be blocked with fibrinogen similar to the receptor on group A, C, and G streptococci (16). No effect of pretreatment with fibrinogen was seen on the binding of

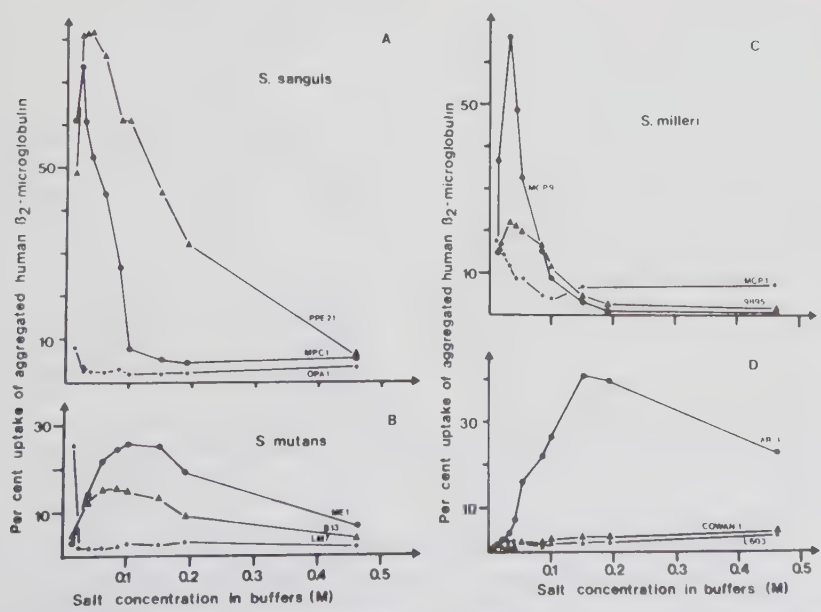


FIG. 4. Effects of variation of salt concentration in buffers on the uptake of labeled aggregated β_2 -microglobulin to oral streptococci (A-C) to a positive control strain AR-1 (a group A *Streptococcus*), and to negative controls *Staphylococcus aureus* Cowan I and *Staphylococcus albus* L603 (D).

aggregated β_2 -microglobulin to oral streptococci.

Effects of pepsin digestion on β_2 -microglobulin binding. *S. mutans* B13 and *S. sanguis* MPC1 and PPE21 were digested with different amounts of pepsin and then tested for binding of aggregated β_2 -microglobulin in 0.15 M PBSAT (Fig. 5A). The receptor for aggre-

gated β_2 -microglobulin on all strains was pepsin sensitive. Almost all reactivity was lost for *S. mutans* B13 and *S. sanguis* MCP1 at pepsin concentrations of 14 and 1.7 $\mu\text{g}/\text{ml}$, respectively, in the buffer.

Effect of formaldehyde treatment on β_2 -microglobulin binding. *S. mutans* B13, and *S.*

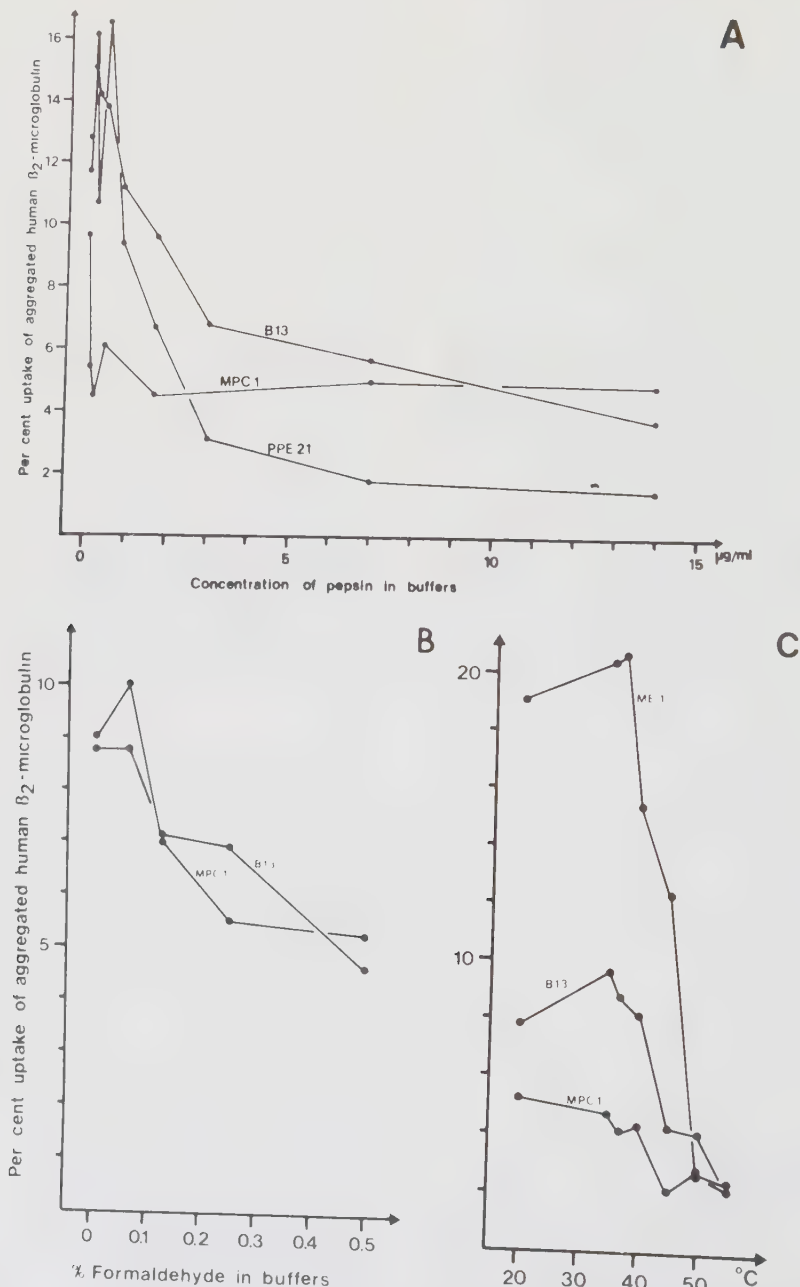


FIG. 5. Effects of pepsin digestion (A), formaldehyde treatment (B), and heat treatment (C) on the uptake of labeled aggregated β_2 -microglobulin to oral streptococci.

sanguis MPC1 were suspended in PBSAT containing formaldehyde from 0 to 0.5%. Both strains showed a decreased affinity for normal aggregated human β_2 -microglobulin after treatment with formaldehyde. At a concentration of 0.5% in the buffers, the levels of reactivity were close to negative control values (Fig. 5B).

Effect of heat treatment on β_2 -microglobulin binding. *S. mutans* B13 and ME1 and *S. sanguis* MPC1 were heat treated before uptake assays for aggregated β_2 -microglobulin. The receptors for the protein on all strains were extremely temperature sensitive. After treatment at 55°C for 10 min, the affinity of all strains for aggregated β_2 -microglobulin was totally lost (Fig. 5C).

DISCUSSION

Aggregated β_2 -microglobulin has shown binding to strains of oral streptococci in previous studies (8). In this study, a larger number of oral streptococci were tested for binding of aggregated β_2 -microglobulin under different buffer conditions with special consideration of possibly important factors in the oral cavity. Bacterial attachment in the oral cavity depends on a wide variety of interactions (9, 10). The importance of any single factor in this complex system of interactions is difficult to evaluate. As compared with serum and other body fluids, saliva is considerably different in its composition and provides an extraordinary environment for bacteria. For example, there is a lower total protein content, hypotonicity, and variations of pH (23). Also, direct and indirect dietary effects on the saliva add factors to the environment of oral bacteria.

When studying binding of aggregated β_2 -microglobulin in 0.15 M PBSAT, 36% of the oral bacterial strains showed affinity for the protein. When comparing groups or serotypes of oral bacteria with binding of aggregated β_2 -microglobulin, no correlations were seen. When saliva rather than 0.15 M PBS was used as the buffer, in assays for uptake of aggregated β_2 -microglobulin, an increase of binding between protein and bacteria was seen (Fig. 1). Comparing oral bacteria and the β_2 -microglobulin-positive group A *Streptococcus* strain AR-1, the increase in affinity of oral streptococci was more than twice the increase for strain AR-1. This phenomenon can be caused by several factors in saliva such as pH, salt concentration, and salivary proteins.

A change in salt concentration of the buffers used affected binding characteristics of different strains in different ways (Fig. 4). Oral streptococci had distinct uptake maxima of aggregated β_2 -microglobulin at low salt concentrations, and the group A *Streptococcus* strain had a maxi-

mum at a higher salt concentration. The maxima for the oral bacteria were close to the salt concentration of saliva, and the maximum of the group A *Streptococcus* strain was close to the salt concentration in serum. Also, there seemed to be a strict species-correlated salt-dependent uptake pattern for the positive strains. Strains of *S. salivarius* all showed a high uptake of aggregated β_2 -microglobulin in 0.066 M PBS, whereas only 2 of the 11 strains were positive in 0.15 M PBS. The different salt concentration-dependent uptake patterns of the oral and the group A streptococci might reflect their choice of natural habitats.

Wide pH variations are not uncommon in saliva, and in dental plaque the pH can be less than 5, particularly after sucrose intake (14). In the test systems presented above, an increase in hydrogen ion concentration below pH 6 in the buffers used significantly increased the affinity between aggregated β_2 -microglobulin and the bacteria tested (Fig. 3). Native β_2 -microglobulin has an isoelectric point at pH 5.4 to 5.7 (21). Below this pH the protein has a positive net charge, whereas most oral bacteria have negative net surface charges (20). Electrostatic forces might, therefore, explain the high nonspecific affinity at low pH. At a pH above the isoelectric point of the protein, affinity between bacteria and protein is dependent upon specific structures with receptor-like characteristics on bacterial surfaces.

Receptors for aggregated human β_2 -microglobulin were first detected in group A, C, and G streptococci (16). These receptors are relatively heat stable in contrast to the extreme heat sensitivity seen for receptors on oral streptococci. The receptors on oral streptococci were sensitive to pepsin and formaldehyde treatment, indicating a protein component in the receptor structure. The difference in salt concentration-dependent uptake of aggregated human β_2 -microglobulin and the earlier described difference in trypsin digestion sensitivity (8, 16) underline major structural differences between receptors for aggregated β_2 -microglobulin in oral streptococci and in group A, C, and G streptococci.

Determination of binding constants between *S. mutans* strain ME1 and aggregated β_2 -microglobulin at pH 7.2 revealed binding structures on bacteria with heterogeneous affinity for the protein. The apparent number of binding structures on each bacterium was low for both the high- and the low-affinity types of receptors. Monomeric β_2 -microglobulin did not bind to oral bacteria in the test system. Group A, C, and G streptococci are highly dependent upon the size of aggregates of β_2 -microglobulin for binding of the protein. Aggregates of a molecular weight of

less than 100,000 show only slight affinity for bacteria (16). Aggregates of human α_1 -microglobulin did not show affinity for oral bacteria in control experiments, indicating that the binding of aggregated β_2 -microglobulin is of a specific character. It is clear from these data that each apparent receptor structure is a measure of a strong synergistic effect obtained through multipoint attachment. When aggregates of the protein are used in binding studies, the primary low-affinity receptors on bacteria together mimic a high-affinity receptor.

The dependency of multipoint attachment for binding of β_2 -microglobulin is of interest considering possible functions in vivo. β_2 -Microglobulin is present on the cell membranes of all nucleated cells, including the epithelial cells of the oral cavity. The arrangement of β_2 -microglobulin on the cell membrane would strongly favor multiple binding, thereby minimizing the effects of soluble β_2 -microglobulin in saliva. This aspect would indicate biological significance of bacterial receptors for β_2 -microglobulin arranged in a macromolecular way on epithelial cell surfaces.

LITERATURE CITED

- Avrameas, S. 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of conjugates for the detection of antigens and antibodies. *Immunochemistry* 6:43-52.
- Berggård, B., L. Björck, R. Cigén, and L. Lögberg. 1980. β_2 -Microglobulin. *Scand. J. Clin. Lab. Invest.* 140: Suppl. 154.
- Berggård, I., and A. G. Bearn. 1968. Isolation and properties of a low molecular weight β_2 -microglobulin occurring in human biological fluids. *J. Biol. Chem.* 243:4095-4103.
- Bratthall, D. 1972. Immunofluorescent identification of *Streptococcus mutans*. *Odontol. Revy* 23:181-196.
- Carlsson, J. 1967. Presence of various types of non-haemolytic streptococci in dental plaque and in other sites of the oral cavity in man. *Odontol. Revy* 18:55-74.
- Carlsson, J. 1968. A numerical taxonomic study of human oral streptococci. *Odontol. Revy* 19:137-160.
- Ekström, B., and I. Berggård. 1977. Human α_1 -microglobulin: purification procedure, chemical and physicochemical properties. *J. Biol. Chem.* 252:8048-8057.
- Ericson, D., D. Bratthall, L. Björck, E. Myhre, and G. Kronvall. 1979. Interactions between human serum proteins and oral streptococci reveal occurrence of receptors for aggregated β_2 -microglobulin. *Infect. Immun.* 25:279-283.
- Gibbons, R. J., and J. van Houte. 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. *Infect. Immun.* 3:567-573.
- Gibbons, R. J., and J. van Houte. 1975. Bacterial adherence in oral microbial ecology. *Annu. Rev. Microbiol.* 29:19-44.
- Gibbons, R. J., J. van Houte, and W. F. Liljemark. 1972. Parameters that effect the adherence of *Streptococcus salivarius* to oral epithelial surfaces. *J. Dent. Res.* 51:424-435.
- Hay, D. J., R. J. Gibbons, and D. M. Spinell. 1971. Characteristics of some high molecular weight constituents with bacterial aggregating activity from whole saliva and dental plaque. *Caries Res.* 5:111-123.
- Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature (London)* 194:495-496.
- Imfeld, Th. 1977. Evaluation of the cariogenicity of confectuary by intra-oral wire-telemetry. *Schweiz. Monatsschr. Zahnheilkd.* 87:437-464.
- Jablon, J. M., and D. D. Zinner. 1966. Differentiation of cariogenic streptococci by fluorescent antibody. *J. Bacteriol.* 92:1590-1596.
- Kronvall, G., E. B. Myhre, L. Björck, and I. Berggård. 1978. Binding of aggregated human β_2 -microglobulin to surface protein structures in group A, C, and G streptococci. *Infect. Immun.* 22:136-142.
- Liljemark, W. F., S. V. Schauer, and C. G. Bloomquist. 1978. Compounds which affect the adherence of *Streptococcus sanguis* and *Streptococcus mutans* to hydroxyapatite. *J. Dent. Res.* 57:373-379.
- Magnusson, I., and Th. Ericson. 1976. Effect of salivary agglutinins on reactions between hydroxyapatite and a serotype c strain of *Streptococcus mutans*. *Caries Res.* 10:273-286.
- Magnusson, I., Th. Ericson, and K. Pruitt. 1976. Effect of salivary agglutinins on bacterial colonization of tooth surfaces. *Caries Res.* 10:113-122.
- Olsson, J., and P.-O. Glantz. 1977. Effect of pH and counter ions on the zeta-potential of oral streptococci. *Arch. Oral Biol.* 22:461-466.
- Poulik, M. D. 1976. β_2 -microglobulin: present status, p. 155. In G. A. Jamieson and T. J. Greenwalt (ed.), *Trace components of plasma: isolation and clinical significance*, vol. 5. Alan R. Liss, New York.
- Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* 51:660-672.
- Shannon, I. L., R. P. Suddick, and F. J. Dowd, Jr. 1974. Saliva: composition and secretion. *Monogr. Oral Sci.* 2:3-42.
- Westergren, G. 1978. Transformation of *Streptococcus sanguis* to a rough colonial morphology with an increased ability to adhere. *Arch. Oral Biol.* 23:887-891.
- Williams, R., and R. J. Gibbons. 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science* 177:697-699.
- Williams, R. C., and R. J. Gibbons. 1975. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. *Infect. Immun.* 11:711-718.

β_2 -MICROGLOBULIN IN SALIVA AND ITS RELATION TO FLOW RATE IN DIFFERENT GLANDS IN MAN

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Summary—The concentration of β_2 -microglobulin (β_2 -m) in parotid saliva and sera from 13 adult hypogammaglobulinaemic subjects and in parotid saliva from 7 normal adults was determined by radioimmunoassay. Parotid and submandibular/sublingual saliva was collected from four normal and one hypogammaglobulinaemic subject in unstimulated and stimulated samples, and concentrations of IgA and β_2 -m were determined. The β_2 -m concentration in parotid saliva from hypogammaglobulinaemic subjects was 0.50 mg/l (0.2–1.1) and in normal adults 0.41 mg/l (0.2–0.9). In parotid saliva, the concentration of β_2 -m was negatively correlated with the flow rate, whereas in submandibular/sublingual a positive correlation was seen. Serum levels of β_2 -m in hypogammaglobulinaemic subjects were within the normal range of healthy adults. Gel filtration of saliva separated β_2 -m from IgA and from high molecular weight agglutinins. β_2 -m was eluted as one single peak.

INTRODUCTION

β_2 -Microglobulin (β_2 -m), a protein with a molecular weight of 11,800, is present on all nucleated cell surfaces representing the light chain of the major histocompatibility antigens. The protein is also present in saliva and other body fluids in a soluble, monomeric form (for review see Berggård *et al.*, 1980; Plesner and Bjerrum, 1980). Previously, we have shown binding of aggregated β_2 -m to several strains of oral streptococci (Ericson *et al.*, 1979; Ericson, Björck and Kronvall, 1980). The monomeric form of the protein will not bind to bacteria and the biological significance of the binding of aggregated β_2 -m to certain bacteria is still unclear. Searching for a significance of salivary β_2 -m, we wished to find out whether the protein is present in a monomeric or polymeric form, and whether it is also associated with other molecules. The distribution of β_2 -m in saliva from different glands and the relation between flow rates and the concentration of the protein was of interest. Salivary samples from subjects with IgA deficiency sometimes also show different agglutination patterns for some oral streptococci (Bratthall and Björckander, 1980). As aggregated β_2 -m can bind to these bacterial species, salivary concentrations of β_2 -m were assayed.

MATERIALS AND METHODS

Collection of saliva

Citrate-stimulated parotid saliva was collected directly from the duct orifices by Lashley cups. There were 13 adult hypogammaglobulinaemic subjects and 7 normal adults. Data of the hypogammaglobulinaemic subjects have been presented by Bratthall and Björckander (1980). The samples were collected into ice-chilled tubes at a secretion rate of approx. 0.4 ml/min and were immediately frozen. Serum

samples from 6 of the hypogammaglobulinaemic subjects were also obtained. To investigate the possible effect of secretion rate on the concentration of β_2 -m in saliva, unstimulated and citric acid-stimulated parotid and submandibular/sublingual saliva samples from 1 hypogammaglobulinaemic subject and from 4 normal adults were collected using the same technique as Bratthall, Gahnberg and Krasse (1978). Submandibular/sublingual saliva was collected by a silicone rubber device, which was placed under the tongue and had a recess in which the duct orifices were placed without obstruction. Saliva was collected using a slight vacuum. In each series, 9 successive samples of approx. 1 ml were collected in polystyrene tubes, 1 before, 4 during, and 4 after the stimulation period. The samples were frozen immediately. There was a one-tube delay between the changes of flow rate and the changes of IgA and β_2 -m concentration as the ducts of the glands, the collection cups and the tubing of left and right side contained approx. one sample volume. Therefore, the secretion rate measured when collecting one sample must be correlated to the β_2 -m and IgA concentrations in the next successive sample.

Gel-filtration of saliva

Ten millilitres of unstimulated parotid saliva were gel-filtered on a Sepharose 2B (2.5 × 38 cm) and on a Sephadex G-200 column (2.5 × 80 cm; see legends to Figs 3 and 4). Also, 0.01 μ g of 125 I-labelled (Hunter and Greenwood, 1962) monomeric human β_2 -m was added to 10 ml of unstimulated parotid saliva samples which gave a radioactivity of 10^5 counts/min/ml saliva. Furthermore 100 μ l of unlabelled β_2 -m (1 mg/ml) was added to 3 ml of unstimulated parotid saliva.

The different samples were separated by gel filtration on Sepharose 2B and Sephadex G-200 columns and eluted with 0.067 M phosphate buffer, pH 7.1

containing 0.02 per cent NaN_3 . Finally, mixed unstimulated parotid and submandibular/sublingual saliva was concentrated 20-fold and gel-filtered on a Sepharose 2B column and eluted with 1 mM phosphate buffer pH 7.4 (with 0.05 M KCl, 1 mM CaCl_2 and 0.1 mM MgCl_2), containing 0.02 per cent NaN_3 . Determination of agglutinating activity in the fractions was performed according to Olsson, Bratthall and Carlén (1981).

Determination of IgA

The concentration of IgA in the saliva samples was determined by single radial immunodiffusion (Mancini, Carbonara and Heremans, 1965) using rabbit anti-human IgA (α -chain, Behringwerke AG, W. Germany) and stabilized human serum (Behringwerke AG) as standards. As the standard consisted of 7 S IgA, the results may be multiplied by a factor of 3 to reflect more closely the mainly dimeric 11 S IgA in saliva (Brandtzaeg, Fjellanger and Gjeruldsen, 1970). Our figures show the readings before this correction.

Determination of β_2 -m

The concentration of β_2 -m in saliva and serum samples was determined by radioimmunoassay (Plesner, Nørsgaard-Petersen and Boenisch, 1975) using ^{125}I -labelled purified human β_2 -m and goat anti-human β_2 -m. Purified human β_2 -m was used as standard. The detection level was less than 1 ng per ml.

Statistical analysis of the data was performed using the Spearman rank correlation as there was a significant difference of the variances between the parameters compared.

RESULTS

Concentration of β_2 -m in saliva

In slightly stimulated parotid saliva from 7 normal adults, the β_2 -m concentration was 0.41 mg/l (0.2–0.9) and in 13 hypogammaglobulinaemic subjects 0.50 mg/l (0.2–1.1). Serum from 6 of the hypogammaglobulinaemic subjects contained 1.9 mg/l (1.0–2.8). The saliva values for these subjects were not statistically correlated to their serum values.

Figures 1 and 2 illustrate mean values of secretion rates, concentrations of β_2 -m and concentrations of

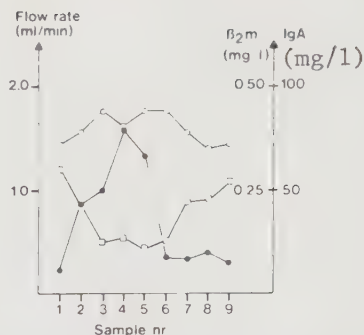


Fig. 2. Relation between flow rate (●—●) concentration of β_2 -m (○—○) and IgA (□—□) in submandibular saliva. Mean values of 4 normal adults.

IgA in parotid and submandibular/sublingual saliva from 4 normal persons without correction for the one-tube delay. The correlation coefficients were calculated after this correction. In parotid saliva from normal adults the concentration of β_2 -m was negatively correlated to the secretion rate ($r_s = -0.88$, $p < 0.005$) and positively correlated to the IgA concentration ($r_s = 0.96$, $p < 0.001$). In submandibular/sublingual saliva, there was a slight positive correlation between secretion rate ($r_s = 0.67$, $p < 0.05$) and β_2 -m concentration, but a negative correlation between IgA and β_2 -m concentration ($r_s = -0.89$, $p < 0.005$). Correlation coefficients between flow rates and IgA concentrations were calculated to be -0.93 ($p < 0.001$) for parotid and -0.74 ($p < 0.025$) for submandibular/sublingual saliva.

The relationship between secretion rate and β_2 -m concentrations in parotid saliva was similar for the normal and for the hypogammaglobulinaemic subject. IgA was not detected in the saliva from this subject.

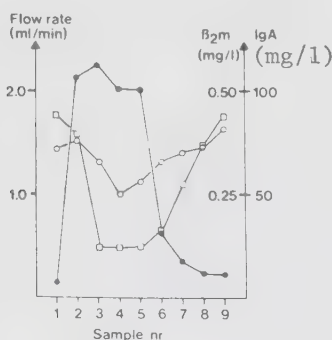


Fig. 1. Relation between flow rate (●—●) concentration of β_2 -m (○—○) and IgA (□—□) in parotid saliva. Mean values of 4 normal adults.

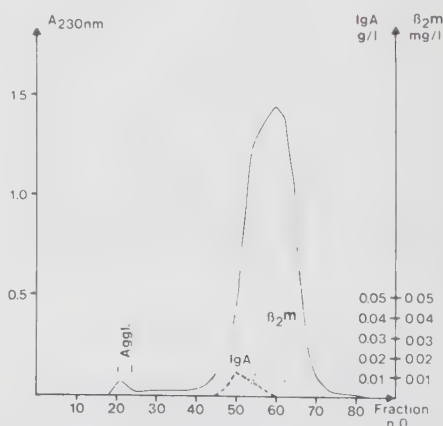


Fig. 3. Elution profile of 10 ml of parotid saliva gel-filtered on a Sepharose 2B column, equilibrated with 0.01 M tris-HCl buffer pH 8.0, containing 0.05 M NaCl and 0.01 per cent NaN_3 . 3 ml fractions were collected with an elution rate of 5 ml per h. Aggl. = agglutinating activity in fractions indicated.

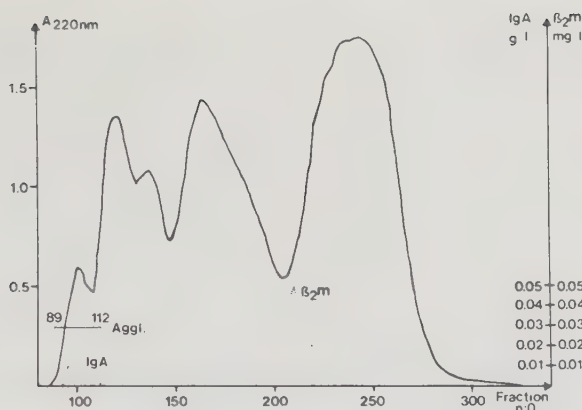


Fig. 4. Elution profile of 10 ml of parotid saliva gel-filtered on a Sephadex G-200 column (2.5 $\times$ 80 cm) equilibrated with a 0.01 M tris-HCl buffer pH 8.0, containing 0.05 M NaCl and 0.01 per cent NaN₃. 1.2 ml fractions were collected with an elution rate of 1.6 ml per h. Aggl. = agglutinating activity in fractions indicated.

Gel filtration of saliva

Gel filtration of saliva showed that β_2 -m emerged as a single peak, separated from IgA and the high molecular weight agglutinins (Figs 3 and 4). The elution position of radiolabelled or unlabelled β_2 -m on Sephadex G-200 (K_{av} = 0.75) corresponds to β_2 -m monomers (Björck and Berggård, 1981).

DISCUSSION

The salivary β_2 -m concentrations were similar in adult normal and hypogammaglobulinaemic subjects. The mean values of β_2 -m in serum and saliva were within the lower ranges of concentrations reported earlier (Evrin *et al.*, 1971; Evrin and Wibell, 1972; Talal *et al.*, 1975). Concentrations varied negatively with the flow rate for parotid saliva. The elution profile on Sepharose 2B and Sephadex G-200 columns indicated that β_2 -m is not closely associated with IgA or high molecular weight salivary agglutinins.

The secretion rate can influence the concentration of IgA (Brandtzaeg, 1971; Bratthall *et al.*, 1978), which we also observed. There was a delay between the increase in flow rate and the decrease in β_2 -m and IgA concentrations in parotid saliva (Fig. 1).

Talal *et al.* (1975) described elevated levels of β_2 -m in parotid saliva from subjects with Sjögren's syndrome. Treatment of them resulted in a decrease of salivary β_2 -m concentration and in an increase of the flow rate. Talal *et al.* found no correlation between β_2 -m concentration and secretion rate in two control subjects. However, in our study in 5 subjects, the β_2 -m concentration in parotid saliva decreased with increased flow rate. The decrease of β_2 -m concentration in parotid saliva from treated subjects with Sjögren's syndrome might have resulted in part from an increased flow rate.

The decrease of β_2 -m in parotid saliva with increased flow rate is perhaps a reflection of a poor

transport of β_2 -m between serum and saliva. The concentration of β_2 -m correlated positively with the flow rate in submandibular/sublingual saliva, in contrast to parotid saliva. The secretion process of the serous acinar cells in the parotid gland is merocrine. On the other hand, in submandibular/sublingual glands, the mucous cells liberate their products by a partly apocrine process, at least when highly stimulated. Even under normal conditions, parts of the cell membrane are shed and excreted with saliva (Tandler and Poulsen, 1976). As β_2 -m is present on cell membranes, β_2 -m will be shed together with the mucous product. This might explain why the β_2 -m concentration in submandibular/sublingual saliva was slightly positively correlated with the secretion rate. β_2 -m should be associated with cell membrane components, if they are shed together. We did not detect such complexes. As the salivary samples gel-filtered on the different columns consisted of either parotid saliva or mixed parotid and submandibular/sublingual collected without stimulation, the concentration of such complexes is perhaps below detection level.

Gel-filtration procedures showed that β_2 -m emerges as one peak. Although our data indicate that β_2 -m was not associated with salivary components, this does not exclude the possibility of β_2 -m being capable of interacting with other salivary molecules *in vivo*, as might occur with IgA and agglutinins (Olsson *et al.*, 1981).

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REFERENCES

- Berggård B., Björck L., Cigén R. and Lögdberg L. 1980. β_2 -Microglobulin. *Scand. J. clin. Lab. Invest.* **40**, Suppl. 154, 13–25.
- Björck L. and Berggård I. 1981. Studies on β_2 -microglobulin in the rabbit. *Molec. Immunol.* **18**, 537–544.

- Brandtzaeg P., Fjellanger I. and Gjeruldsen S. T. 1970. Human secretory immunoglobulins I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins. *Scand. J. Haematol. Suppl.* No. 12.
- Brandtzaeg P. 1971. Human secretory immunoglobulins—VII. Concentrations of parotid IgA and other secretory proteins in relation to the rate of flow and duration of secretory stimulus. *Archs oral Biol.* **16**, 1295–1310.
- Bratthall D. and Björkander J. 1980. Bacteria and oral fluid components: report of the oral condition in hypogammaglobulinaemic patients. In: *The Borderland between Caries and Periodontal Disease II* (Edited by Lehner T. and Cimasoni G.) pp. 159–173. Academic Press, London.
- Bratthall D., Gahnberg L. and Krasse B. 1978. Method for detecting IgA antibodies to *Streptococcus mutans* serotypes in parotid saliva. *Archs oral Biol.* **23**, 843–849.
- Ericson D., Bratthall D., Björck L., Myhre E. and Kronvall G. 1979. Interactions between human serum proteins and oral streptococci reveal occurrence of receptors for aggregated β_2 -microglobulin. *Infect. Immun.* **25**, 279–283.
- Ericson D., Björck L. and Kronvall G. 1980. Further characteristics of β_2 -microglobulin binding to oral streptococci. *Infect. Immun.* **30**, 117–124.
- Evrin P. E., Peterson P. A., Wide L. and Berggård I. 1971. Radioimmunoassay of β_2 -microglobulin in human biological fluids. *Scand. J. clin. Lab. Invest.* **28**, 439–443.
- Evrin P. E. and Wibell L. 1972. The serum levels and urinary excretion of β_2 -microglobulin in apparently healthy subjects. *Scand. J. clin. Lab. Invest.* **29**, 69–74.
- Hunter W. M. and Greenwood F. C. 1962. Preparation of Iodine-131 labelled human growth hormone of high specific activity. *Nature* **194**, 495–496.
- Mancini G., Carbonara A. and Heremans J. 1965. Immunochemical quantitation of antigen by single radial immunodiffusion. *Immunochemistry* **2**, 235–254.
- Olsson J., Bratthall D. and Carlén A. 1981. Association between bacterial agglutinins and immunoglobulin A in human saliva. *Acta odont. scand.* **39**, 61–66.
- Plesner T., Nörsgaard-Petersen B. and Boenisch T. 1975. Radioimmunoassay of β_2 -microglobulin. *Scand. J. clin. Lab. Invest.* **35**, 729–735.
- Plesner T. and Bjerrum O. J. 1980. Distribution of "free" and HLA-associated human β_2 -microglobulin in some plasma membranes and biological fluids. *Scand. J. Immunol.* **11**, 341–351.
- Talal N., Grey H. M., Zvaifler N., Michalski J. P. and Daniels T. E. 1975. Elevated salivary and synovial fluid β_2 -microglobulin in Sjögren's syndrome and rheumatoid arthritis. *Science* **187**, 1196–1198.
- Tandler B. and Poulsen J. H. 1976. Fusion of the envelope and mucous droplets with the luminal plasma membrane in acinar cells of the cat submandibular gland. *J. Cell Biol.* **68**, 775–781.

Agglutination of Streptococcus mutans by Low-Molecular-Weight
Salivary Components: Effect of β_2 -Microglobulin

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ABSTRACT

Radiolabeled monomeric human β_2 -microglobulin (β_2 m) was tested for binding to Streptococcus mutans strains in buffers containing 1 mM calcium (Ca^{++}). Binding was seen to strains with a previously established binding capacity of aggregated β_2 m. Monomeric β_2 m agglutinated β_2 m-binding strains when Ca^{++} was present. At Ca^{++} concentrations of 1.4 mM, 0.032 μg per ml of monomeric β_2 m caused bacterial agglutination.

Parotid saliva was gel filtered on a Sephadex G-75 column, and low-molecular-weight fractions containing β_2 m could agglutinate S. mutans cells. Five of six strains which could bind β_2 m were agglutinated by these fractions, but only one of five nonbinding strains was. All strains tested were agglutinated by void volume fractions.

A new method for the measurements of turbidity in bacterial agglutination inhibition experiments with parotid saliva was used. Suspensions containing parotid saliva, bacteria, and control serum were directly compared in a spectrophotometer with test suspensions containing goat anti-human β_2 m, bacteria, and saliva. Thus, the spectrophotometer directly read the difference in agglutination of the two suspensions, and the result was presented as one curve by the recorder. Agglutination of five β_2 m-binding strains of S. mutans was inhibited or decreased by the addition of goat anti-human β_2 m as compared with control serum. The agglutination of two β_2 m-nonbinding strains and one with variable binding was not inhibited. Thus, salivary β_2 m may contribute to agglutination of S. mutans cells in parotid saliva.

INTRODUCTION

Oral streptococci can be agglutinated by various salivary molecules including high-molecular-weight agglutinins (13, 17-19, 27, 31), secretory immunoglobulin A (IgA) (20, 24, 33), blood group reactive substances (16, 34), and lysozyme (30). Generally, the agglutination phenomenon of bacteria in saliva has been considered to be a host defense system for the disposal of bacteria (32-34). However, the bacterial agglutinating factors can also serve as adherence-promoting factors in the pellicle (23-25), and the formation of small aggregates may increase the number of bacteria binding to saliva-coated hydroxyapatite (23). Even though bacterial binding of a particular molecule does not result in agglutination, the bacterial surface properties may be altered (26) and their colonization abilities may be changed.

It seems unlikely that one bacterial binding or agglutinating component is solely responsible for the clearance or attachment of a specific bacterium; several probably contribute to the resulting effect (15, 32). There have also been reports that indicate interaction between high-molecular-weight agglutinins and secretory IgA, which could both have affinity for the same species (9, 27). This makes the functional picture more complex. Therefore, the importance of bacterial binding by a single isolated factor is not easy to demonstrate. Nevertheless, evaluation of the effect of single factors is necessary.

We have earlier demonstrated that aggregated β_2 -microglobulin (β_2 m) can interact with strains of oral streptococci (10, 12). β_2 m is a low-molecular-weight protein occurring on surfaces of all nucleated cells as the light chain of the major histocompatibility antigens (for a review, see reference 3). β_2 m is also present in saliva and other body fluids (2, 3, 11, 28). Previous studies have shown that aggregates, but not monomers, of β_2 m bind to bacteria (12, 21). Aggregation of β_2 m to high-molecular-weight complexes strongly increases the avidity of the interaction between bacteria and the β_2 m complexes. The complexes then can bind to low-affinity receptors (10, 12). The need for a multipoint attachment has been considered significant in the binding of bacteria to cell surfaces (5), where β_2 m seems

to be present as repetitive units. In saliva, however, high-molecular-weight complexes containing β_2m have not been found, but β_2m is present as monomers (11). One aim of this study was to learn whether binding of β_2m monomers to Streptococcus mutans cells can be demonstrated. To do this, the influence of calcium ions (Ca^{++}) on the binding of the protein to bacteria was analyzed, since some bacteria-binding salivary agglutinins demand Ca^{++} for interaction with bacteria (9, 17, 31), and others do not (22, 30, 33).

Another aim was to investigate whether β_2m monomers, the form present in saliva, could agglutinate strains of S. mutans and whether salivary fractions containing β_2m could agglutinate S. mutans cells. The effect of addition of anti- β_2m antibodies on the agglutination of S. mutans strains in parotid saliva was also studied.

MATERIAL AND METHODS

Bacterial cultures and growth conditions

Bacterial strains that were analysed previously for binding aggregated β_2m were selected (10, 12). Binding of aggregated β_2m was scored as +, - or +/- (see Table 1, footnote a) in the following strains: S. mutans AHT (+) and 3720 (+) (serotype a); BHT (-) (serotype b); P8 (-), KPSK 2 (+/-), 1449 (-) and NCTC 10449 (+) (serotype c); ME1 (+) (serotype d); LM7 (-) (serotype e); OMZ 175 (+) (serotype f); and K1 (-), OMZ65 (+) (serotype g). Bacterial cultures were maintained on blood agar plates and transferred to tubes containing 10 ml of dialyzed yeast extract medium (7). After incubation in anaerobic jars overnight, in 95% N_2 -5% CO_2 , the bacteria were washed twice in phosphate-buffered saline (0.01 M phosphate, 0.15 M NaCl (pH 7.0); PBS) and suspended to an optical density (OD) of 1.50 at 700 nm in a Beckman model 35 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.).

Binding studies

Binding of monomeric ^{125}I -labeled human β_2m to bacteria was tested as described earlier, with some exceptions (10, 12). Briefly, 200 μ l of a suspension of 10^9 bacteria per ml in KCl buffer was incubated with 25 μ l (ca 0.3 μ g giving a radioactivity of 10,000 cpm) of radiolabeled

human β_2m (these mixtures did not contain Tween 20 or human serum albumin (HSA) as in the earlier method). After incubation for 60 min, the suspension was washed with 2 ml of KCl buffer with 0.05% Tween and centrifuged. The radioactivity in the pellet was measured and compared with the radioactivity in 25 μ l of labeled protein solution. In another experiment, 0.05 % Tween 20 was added to the 25 μ l of protein solution before incubation with the bacterial suspensions.

Saliva and gel filtration

Parotid saliva from two subjects was stimulated with citric acid to a flow rate of approximately 0.4 ml/min and collected in modified Lashley cups. Samples were used immediately for gel filtration. A 7-ml sample of saliva was fractioned on a Sephadex G-75 column (Pharmacia Fine Chemicals, Sweden) (2.5 by 42 cm) and eluted with 1 mM phosphate buffer (pH 6.95) (with 0.05 M KCl, 1 mM $CaCl_2$, and 0.1 mM $MgCl_2$; KCl-buffer) containing 0.02% NaN_3 . Fractions (4.9 ml each) were collected at an elution rate of 14.6 ml/h, and the absorbance at 280 nm was measured in the spectrophotometer. The elution positions of blue dextran 2000 (Pharmacia) and hen egg white lysozyme (HEWL) (E. Merck AG, Darmstadt, Federal Republic of Germany) were determined on the G-75 column.

IgA analysis

The concentration of IgA in every second fraction from the Sephadex G-75 column was measured by an immunobead enzyme-linked immunosorbent assay (6).

β_2m analysis

The concentration of β_2m in saliva fractions from the Sephadex G-75 column was determined by a radioimmunoassay, using ^{125}I -labeled purified human β_2m and goat anti-human β_2m ($G\beta_2m$) (29). Preparation procedures and properties of $G\beta_2m$ are given extensively elsewhere (4). The elution position of β_2m on the Sephadex G-75 column was also determined by gel filtration of monomeric human ^{125}I -labeled β_2m .

Amylase analysis

The presence of amylase in fractions was assayed by immunodiffusion in agarose gel (1% ME agarose (FMC Corp., Rockland, Maine) in 0.02 M

barbital buffer (pH 8.6)) against rabbit anti-human secretory amylase (Nordic, London; batch 9-1177).

Agglutination assays

Fifty μ l of gel filtration fractions was mixed with 25 μ l of bacterial suspension in a microtiter plate (Dynatech M129A, Dynatech Deutschland, Federal Republic of Germany). Bacterial agglutination was determined after 90 min with a stereomicroscope at 10x magnification by comparing β_2 m-containing and β_2 m-free suspensions. The degree of agglutination was rated ++, indicating heavy agglutination; +, indicating weak but definite agglutination; or -, indicating no agglutination.

Human β_2 m was isolated from urine samples as previously described (2) and was aggregated with glutaraldehyde (1). Monomeric and aggregated β_2 m were diluted in KCl buffer, and 50 μ l samples were used for agglutination assays as described above, except that agglutination was determined after 3 to 5 hours. Different volumes of 10 mM solutions of CaCl_2 in distilled water were added to incubation mixtures in some experiments to determine whether the divalent cation Ca^{++} would influence agglutination. Lysozyme has a molecular weight somewhat higher than that of β_2 m and has been reported to agglutinate oral streptococci (30). HEWL was therefore included as a control in agglutination assays.

Agglutination inhibition experiments

The five strains of S. mutans capable of binding aggregated β_2 m were tested for agglutination by freshly collected parotid saliva mixed with $\text{GA}\beta_2$ m or preimmune control serum (CS) from the same animal respectively. Two nonbinding strains and one with a variable binding were also tested. First, 10 μ l of $\text{GA}\beta_2$ m or CS diluted 1:10 in PBS was added to 150 μ l of parotid saliva diluted 1:2 in PBS, in Hellma Suprasil Quartz microcells (Hellma, Müllheim/Baden, Federal Republic of Germany) at 37°C. After 50 min, 250 μ l of bacterial suspension was added. Agglutination was followed at 700 nm in a Beckman model 35 spectrophotometer with a four-position manual sample changer at 37°C. Positive controls contained 150 μ l of parotid saliva diluted 1:2 in PBS and a 250 μ l bacterial suspension. Negative controls contained PBS instead of saliva.

Cells containing positive control or CS were placed in reference positions and cells containing negative control or GA β_2 m were placed in sample positions in the spectrophotometer (Fig. 1b). Agglutination in the positive control but not the negative control was read as an increase in absorbance. Similarly, when GA β_2 m-containing cells were read against CS-containing cells, a faster agglutination rate in the CS-containing cells was read as an increase of absorbance. If the agglutination rate was identical in the cells, no increase of absorbance was seen (Fig. 1c). For comparison, three of the strains were assayed according to Ericson et al. (13), using cells containing PBS in reference position (Fig. 1a, 2a, 3a).

RESULTS

Binding experiments

Radiolabeled monomeric β_2 m bound to three strains that had also bound aggregated β_2 m and to one strain that had shown a variable affinity for aggregated β_2 m (Table 1). The addition of 0.05% Tween 20 totally inhibited binding of all strains tested (Fig. 4).

Parotid saliva fractioned on a Sephadex G-75 column eluted as four peaks (Fig. 5). Salivary IgA eluted in the void volume, and β_2 m eluted in peak 3. The maximum concentration of β_2 m was 4.7 μ g/l. In another control column run, radiolabeled human monomeric β_2 m eluted at the same position. The molecular weight marker blue dextran 2000 eluted with void volume, and HEWL eluted in fractions 32 to 39. Amylase was detected in fractions 34 to 49 (peaks 2 and 3). All strains tested were agglutinated by fractions from peak 1. Of six strains that bound aggregated β_2 m, five strains were agglutinated by fractions from peak 3, and of five negative strains, only S. mutans BHT was agglutinated in one of two assays (Table 1). Agglutination by void volume fractions was mostly seen after 30 min, but in low-molecular-weight fractions it was not until after 60 to 90 min.

Preparations of aggregated human β_2 m agglutinated all three strains that had bound aggregated human β_2 m, also in PBS free of Ca $^{++}$. For S. mutans ME1, showing the highest affinity for aggregated β_2 m, the lowest agglutinating concentration was 0.02 μ g/ml. Three nonbinding

controls were also not agglutinated. Monomeric human β_2m agglutinated strains that bound the aggregated form of the protein. S. mutans ME1 was agglutinated at a concentration of 0.032 $\mu g/ml$.

HEWL agglutinated all six strains tested at concentrations from 39 to 200 $\mu g/ml$ (Table 1). S. mutans BHT agglutinated at the lowest concentration. None of the bacteria tested were agglutinated by G-75 fractions at the elution position of HEWL.

The agglutination of eight strains of S. mutans strains in parotid saliva with addition of $GA\beta_2m$ or CS was followed spectrophotometrically. Figure 2a illustrates the agglutination of the β_2m -binding strain ME1. $GA\beta_2m$ totally inhibited agglutination (curve 2) and CS gave a minor inhibition (curve 3). Figure 2b shows the same events as in Fig. 2a, but the assay was performed by the method for Fig. 1b. In this assay, only the difference between the agglutinations in Fig. 2a was measured. The numbers (1 to 4) in Fig. 2a correspond to the same numbers in Fig. 2b. The curve 1-4 expresses the change of the absolute value of the difference in OD between curve 1 and 4 in Fig. 2a. Thus, the suspension that contained saliva and bacteria agglutinated faster than did the suspension with PBS and bacteria. Similarly, the curve 2-3 increases, showing that $GA\beta_2m$ inhibited agglutination as compared with CS.

The β_2m -non-binding LM7 was tested (Fig. 3a and b). $GA\beta_2m$ did not inhibit agglutination as compared with CS (Fig. 3a). In Fig. 3b, the curve 2-3 does not increase, which shows that bacterial suspensions with parotid saliva and CS or $GA\beta_2m$ agglutinated almost identically. The curve 1-4 increases, which shows that the strain LM7 was agglutinated by parotid saliva but not by PBS.

The agglutination of the five strains tested that could bind aggregated β_2m was inhibited by $GA\beta_2m$. ME1 was strongest inhibited. The nonbinding and the variably binding strains were not affected differently by $GA\beta_2m$ or CS.

DISCUSSION

It was demonstrated that β_2m monomers also can bind to S. mutans strains, and not only in the aggregated form as earlier assumed (10, 12, 21). As with some other salivary molecules (9, 17, 31), binding of β_2m to bacteria seemed to require presence of Ca^{++} . Previous β_2m -binding studies were performed with Ca^{++} -free buffers containing Tween 20 or HSA. When a KCl buffer with 1 mM Ca^{++} and without Tween 20 or HSA was used, monomeric β_2m was bound to the four strains that also bound aggregated β_2m , but not to the negative strains. HSA or Tween is often used to prevent binding to reaction vessels and might interfere with low affinity interactions. Even though a higher adsorption of β_2m to tubes and negative control bacteria was found in this study, there was a clear difference between controls and β_2m -binding strains. The presence of Tween or HSA might mask the presence of low-affinity receptors, but it does not seem to interfere with the higher avidity reactions between β_2m aggregates and bacteria (10, 12, 21).

Purified preparations of human monomeric and aggregated β_2m agglutinated the strains that bound the protein but not the negative strains. As little as 0.032 μg of monomeric β_2m per ml induced agglutination. However, in gel-filtered saliva fractions causing agglutination, the maximum concentration of β_2m was 0.0047 $\mu g/ml$. Purification procedures and storage might have decreased the affinity of the purified β_2m to bacteria as compared with freshly tested salivary β_2m . The concentration of β_2m in unfractionated saliva is 0.2 to 0.9 $\mu g/ml$ (11), which is several times the concentration needed for agglutination.

Amylase, having a molecular weight of approximately 5.5×10^5 , eluted in fractions with lower molecular weights. This has been ascribed to interaction between the Sephadex gel and amylase (14), and might explain the lower absorption at 280 nm by higher-molecular-weight fractions.

Among the Sephadex G-75 fractions, all bacteria were agglutinated by void volume fractions. The lower-molecular-weight fractions not containing IgA or high-molecular-weight agglutinins, but containing

β_2m , could agglutinate five of six strains that readily bound β_2m . The low concentration of β_2m in the fractions may explain the failure of one strain to agglutinate. The slower agglutination seen in the lower-molecular-weight fractions indicates that agglutinating factors are either not as strong or not as concentrated as those seen in the void volume.

Strain BHT was the only β_2m -nonbinding strain that was variably agglutinated by fractions containing β_2m . This strain has been reported to show a high affinity for lysozyme (30). However, since HEWL eluted in fractions just before peak 2 on the G-75 column, and these fractions never agglutinated bacteria, lysozyme is here probably not responsible for the agglutination of strain BHT. Also, binding and agglutination by β_2m did not correlate with agglutination by lysozyme for the strains tested.

The method presented in this paper had some advantages as compared with previously described methods (13) for turbidimetical measurements of bacterial agglutination inhibition experiments. In measuring the effect of added antiserum against a specific salivary molecule, a CS often also interferes with agglutination. This effect is often not interesting to study per se, but it has to be subtracted from the phenomenon one wishes to study. In measuring only the differences between agglutination in saliva treated with antiserum and with CS, the effect of CS was subtracted in the spectrophotometer. Thus, small differences in agglutination could be detected, and specific inhibition by the antiserum was directly revealed. Also, as the agglutination in control and test cuvettes were measured against each other, and not against a buffer control, only half the number of cuvettes was used as compared with the method described for Fig. 1a, 2a and 3a. The results obtained with this method correlate with those obtained by other methods (13) (Fig. 2a and b, 3a and b).

For the highest affinity strain S. mutans ME1, agglutination was inhibited almost completely when $GA\beta_2m$ was added (Fig. 2a and b). Agglutination of other β_2m -binding strains tested was not so strongly affected by $GA\beta_2m$. Since agglutination of bacteria can be caused by several factors in saliva, one would expect that interfering with one factor with antibodies would not totally inhibit agglutination.

However, this was seen with strain ME1. As ME1 showed the highest affinity of the strains tested, it might have been possible that complexes between antibody and β_2m were absorbed onto the bacteria without causing agglutination. Receptors for other bacterial agglutinating factors may then have been blocked sterically.

The fact that several factors in saliva interact with the indigenous flora is perhaps an important feature of the bacteria. Maybe it is important for the indigenous flora to have several ways to interact with host substances, instead of one strong interaction that might be more vulnerable. Microheterogeneities among salivary molecules and thus many interaction sites have been suggested to provide "zip codes" for disposal or attachment of microorganisms (32). A favorable combination of surface receptors on the bacteria then would be the key to attachment. It is therefore important to clarify all types of interactions between bacteria and salivary factors to be able to understand better the oral ecology. β_2m interacts with hydroxapatite (8) and with some strains of oral bacteria (10, 12). β_2m also participates in the saliva-induced agglutination of these strains. Even though S. mutans can agglutinate in salivary fractions without β_2m , binding of β_2m seems to be of significance for agglutination of these strains.

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LITERATURE CITED

- 1 Avrameas, S. 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* 6:43-52.
- 2 Berggård, I., and A.G. Bearn. 1968. Isolation and properties of a low molecular weight β_2 -globulin occurring in human biological fluids. *J. Biol. Chem.* 243: 4095-4103.
- 3 Berggård, B., L. Björck, R. Cigén, and L. Lögdberg. 1980. β_2 -microglobulin. *Scand. J. Clin. Lab. Invest.* 40, Suppl. 154., 13-25.
- 4 Björck, L., R. Cigén, B. Berggård, B. Löw, and I. Berggård. 1977. Relationships between β_2 -microglobulin and alloantigens coded for by the major histocompatibility complexes of the rabbit and the guinea pig. *Scand. J. Immunol.* 6:1063-1069.
- 5 Björck, L., S. Tylewska, T. Wadström, M. Söderström, and G. Kronvall. 1982. β_2 -microglobulin and its relation to streptococcal M protein and adherence, pp 220-222. In: S.E. Holm and P. Christensson (eds.) Basic concepts of streptococci and streptococcal diseases. Reedbooks Ltd, Chertsey, England.
- 6 Bratthall, D., and R.P. Ellen. 1982. Determination of immunoglobulin A in saliva by immunobead enzyme-linked immunosorbent assay: comparison with single radial immunodiffusion. *J. Clin. Microbiol.* 16:766-769.
- 7 Carlsson, J., E. Newbrun, and B. Krasse. 1969. Purification and properties of dextranucrase from *Streptococcus sanguis*. *Arch. Oral Biol.* 14:469-478.
- 8 Curry, R., J. Thöen, and R.P. Messner. 1981. Rapid semi-quantitative isolation of beta-2-microglobulin from urine. *J. Immun. Methods.* 47:365-373.
- 9 Eggert, F.M. 1980. The nature of secretory agglutinins and aggregating factors. IV. Complexing between non-mucin glycoproteins, immunoglobulins and mucins in human saliva and amniotic fluid. *Int. Arch. Allergy Appl. Immunol.* 62:46-58.
- 10 Ericson, D., L. Björck, and G. Kronvall. 1980. Further characteristics of β_2 -microglobulin binding to oral streptococci. *Infect. Immun.* 30:117-124.
- 11 Ericson, D., D. Bratthall, L. Björck, and G. Kronvall. 1982. β_2 -microglobulin in saliva and its relation to flow rate in different glands in man. *Arch. Oral Biol.* 27:679-682.
- 12 Ericson, D., D. Bratthall, L. Björck, E. Myhre, and G. Kronvall. 1979. Interactions between human serum proteins and oral streptococci reveal occurrence of receptors for aggregated β_2 -microglobulin. *Infect. Immun.* 25:279-283.
- 13 Ericson, Th., K. Pruitt, and H. Wedel. 1975. The reaction of salivary substances with bacteria. *J. Oral Pathol.* 4:307-323.

- 14 Gelotte, B. 1964. Separation of pancreatic enzymes by gel filtration. *Acta Chem. Scand.* 18:1283-1291.
- 15 Gibbons, R.J., E.C. Moreno, and I. Etherden. 1983. Concentration-dependent multiple binding sites on saliva-treated hydroxyapatite for Streptococcus sanguis. *Infect. Immun.* 39:280-289.
- 16 Gibbons, R.J., and J.V. Qureshi. 1978. Selective binding of blood group-reactive salivary mucins by Streptococcus mutans and other oral organisms. *Infect. Immun.* 22:665-671.
- 17 Gibbons, R.J., and D.M. Spinell. 1970. Salivary-induced aggregation of plaque bacteria, pp 207-216: In Dental plaque. W.D. McHugh (ed) Livingstone Ltd., Edinburgh, England.
- 18 Hay, D.I., R.J. Gibbons, and D.M. Spinell. 1971. Characteristics of some high molecular weight constituents with bacterial aggregating activity from whole saliva and dental plaque. *Caries Res.* 5:111-123.
- 19 Kashket, S., and C.G. Donaldson. 1972. Saliva-induced aggregation of oral streptococci. *J. Bacteriol.* 112:1127-1133.
- 20 Kilian, M., K. Roland, and J. Mestecky. 1981. Interference of secretory immunoglobulin A with sorption of oral bacteria to hydroxyapatite. *Infect. Immun.* 31:942-951.
- 21 Kronvall, G., E.B. Myhre, L. Björck, and I. Berggård. 1978. Binding of aggregated human β_2 -microglobulin to surface protein structure in group A, C and G streptococci. *Infect. Immun.* 22:136-142.
- 22 Levine, M.J., M.C. Herzberg, M.S. Levine, S.A. Ellison, M.W. Stinson, H.C. Li, and T. van Dyke. 1978. Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycoproteins with Streptococcus sanguis and Streptococcus mutans. *Infect. Immun.* 19:107-115.
- 23 Liljemark, W.F., C.G. Bloomquist, and G.R. Germaine. 1981. Effect of bacterial aggregation on the adherence of oral streptococci to hydroxyapatite. *Infect. Immun.* 31:935-941.
- 24 Liljemark, W.F., C.G. Bloomquist, and J.C. Ofstehage. 1979. Aggregation and adherence of Streptococcus sanguis: role of human salivary immunoglobulin A. *Infect. Immun.* 26:1104-1110.
- 25 Magnusson, I., and Th. Ericson. 1976. Effect of salivary agglutinins on reactions between hydroxyapatite and a serotype c strain of Streptococcus mutans. *Caries Res.* 10:273-286.
- 26 Miörner, H., E. Myhre, L. Björck, and G. Kronvall. 1980. Effect of specific binding of human albumin, fibrinogen, and immunoglobulin G on surface characteristics of bacterial strains as revealed by partition experiments in polymer phase systems. *Infect. Immun.* 29:879-885.
- 27 Olsson, J., D. Bratthall, and A. Carlén. 1981. Association between bacterial agglutinins and immunoglobulin A in human saliva. *Acta Odontol. Scand.* 39:61-66.

- 28 Plesner, T., and J. Bjerrum. 1980. Distribution of free and HLA-associated human β_2 -microglobulin in some plasma membranes and biological fluids. *Scand. J. Immunol.* 11:341-351.
- 29 Plesner, T., B. Nørgaard-Pedersen, and T. Boenisch. 1975. Radio-immunoassay of β_2 -microglobulin. *Scand. J. Clin. Lab. Invest.* 35:729-735.
- 30 Pollock, J.J., V.J. Iacono, H. Goodman Bicker, B.J. MacKay, L. I. Katona, L.B. Taichman, and E. Thomas. 1976. The binding, aggregation and lytic properties of lysozyme, pp 325-352. In H. M. Stiles, W.J. Loesche, and T.C O'Brien (eds.) *Microbial Aspects of Dental Caries*, vol II.
- 31 Rundegren, J., and Th. Ericson. 1981. Effect of calcium on reactions between a salivary agglutinin and a serotype c strain of *Streptococcus mutans*. *J. Oral Pathol.* 10:269-275.
- 32 Tabak, L.A., M.J. Levine, I.D. Mandel, and S.A. Ellison. 1982. Role of salivary mucins in the protection of the oral cavity. *J. Oral Pathol.* 11:1-17.
- 33 Williams, R.C., and R.J. Gibbons. 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science* 177:697-699.
- 34 Williams, R.C., and R.J. Gibbons. 1975. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. *Infect. Immun.* 11:711-718.

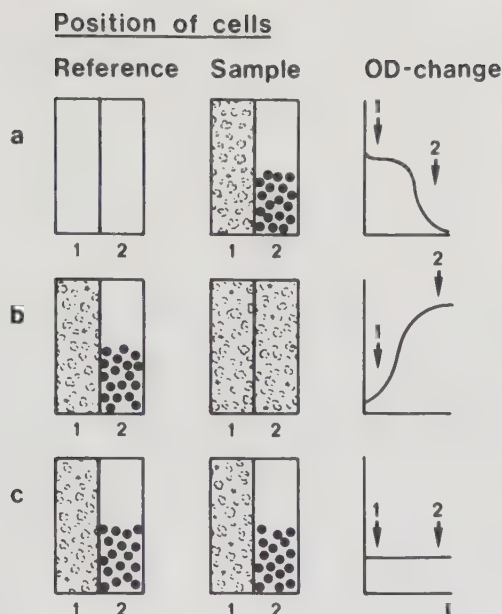


FIG 1. Placement of cells for studying agglutination of bacteria in the spectrophotometer. (a) If bacteria agglutinate in the sample position cell, a decrease in OD at $t=2$ will be seen as compared with buffer-containing cell in reference position. (b) If bacteria agglutinate in the reference position cell, but not in the sample position cell, the OD will increase from 0 at $t=1$ (if both cells had the same OD) to a value showing the difference in OD between the cells at $t=2$. (c) No change in OD will be seen if bacteria behave the same in sample and reference positions.

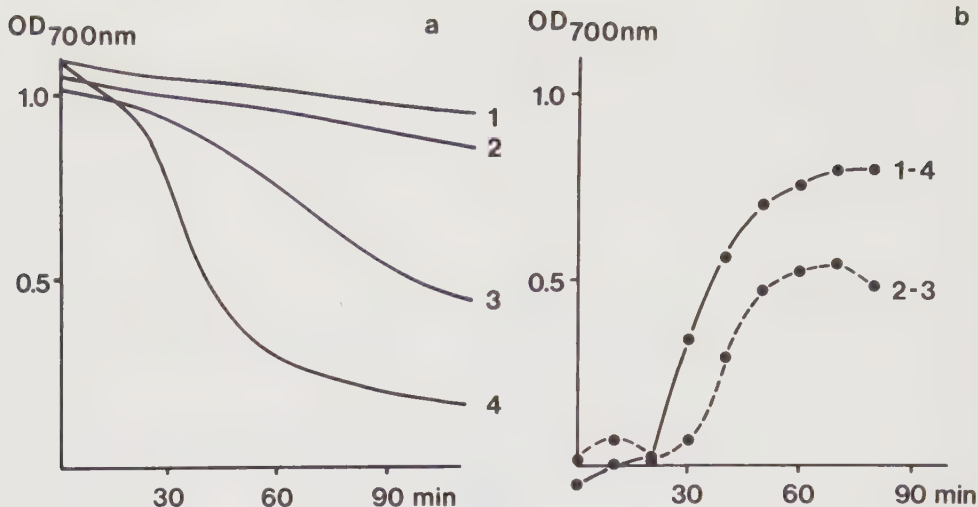


FIG 2. Agglutination of *S. mutans* ME1, (a β_2 m-binding strain) in parotid saliva and buffer. (a) Curves: 1, bacteria and buffer (positive control); 2, bacteria, saliva and GA β_2 m; 3, bacteria, saliva, and CS; 4, bacteria and saliva (positive control). (b) According to FIG 1b, the increasing OD value shows that agglutination is taking place in cells in control position (as compared with in the cell in sample position). Curves: 1-4, sample-positioned cell contains ME1 and buffer, and the reference-positioned cell contains ME1 and saliva; 2-3, the sample-positioned cell contains bacteria, saliva, and GA β_2 m; the reference-positioned cell contains bacteria, saliva and CS. The increase in the OD value indicates that GA β_2 m inhibits or decreases agglutination as compared with CS.

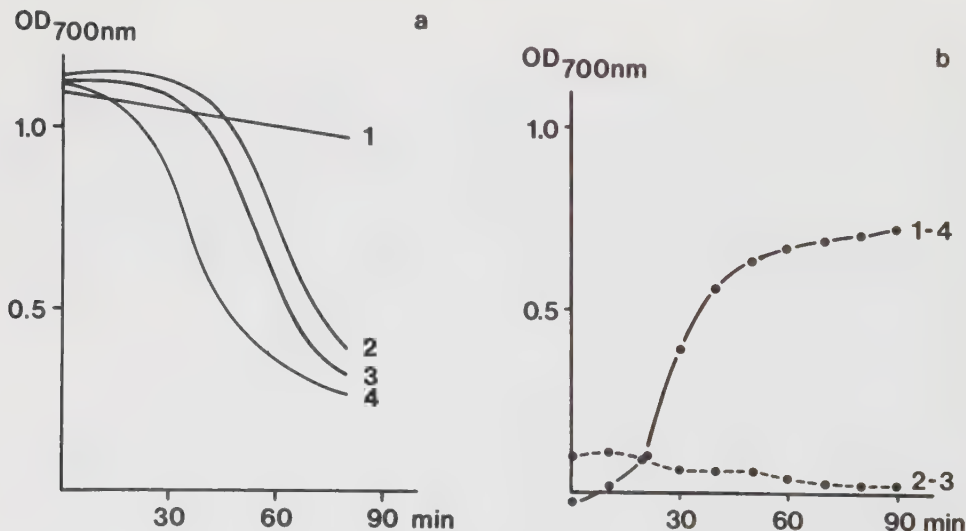


FIG 3. Agglutination of *S. mutans* LM7 (a β_2 m-nonbinding strain). Symbols are the same as in FIG 2.

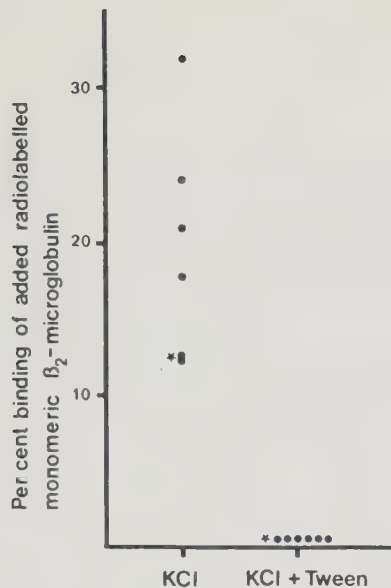


FIG 4. Binding of ^{125}I radiolabeled monomeric $\beta_2\text{m}$ to strains of *S. mutans* expressed as the percentage of added $\beta_2\text{m}$ with and without 0.05% Tween 20 in reaction mixtures. Symbols: $\bullet$, binding to bacterial strains; *, binding to control tubes.

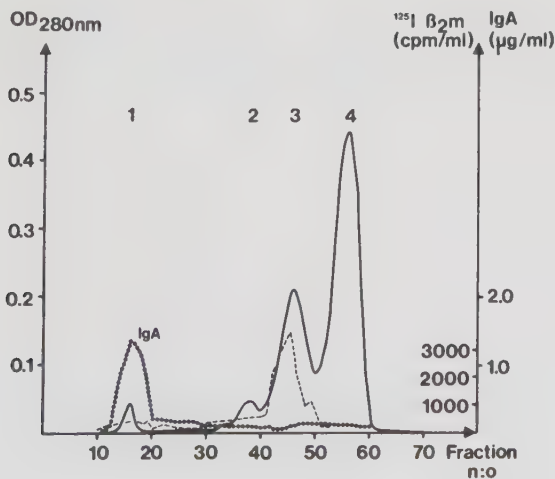


FIG 5. Elution profile of 7 ml of parotid saliva separated on a Sephadex G-75 column. Symbols: —, absorbance at 280 nm; ---, radioactivity corresponding to ^{125}I -labeled human monomeric $\beta_2\text{m}$; ..., IgA concentrations as measured by immunobead assay.

TABLE 1. Binding of aggregated radiolabeled human β_2m by S.mutans in relation to agglutination by β_2m , salivary fractions, and HEWL, and in relation to uptake of radiolabeled monomeric β_2m

Parameter	Rate of binding of aggregated radiolabeled β_2m in previous studies ^a		
	+	-	+/-
Agglutination by peak from the Sephadex G-75	5/6	1 ^b /5	0/1
Uptake of radiolabeled monomeric β_2m	3/3	0/2	1/1
Agglutination by monomeric β_2m	3/3	0/2	1/1
Agglutination by aggregated β_2m	3/3	0/3	1/1
Agglutination by lysozyme	3/3	3/3	1/1

^a Expressed as number of reactive strains/total number of strains tested. Symbols: +, Binding of aggregated β_2m ; -, no binding of aggregated β_2m ; +/-, variable binding of aggregated β_2m .

^b Strain BHT.

Salivary interactions with homologous and heterologous strains of
oral streptococci and with epithelial cells.

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Abstract - Twenty strains of oral streptococci representing the species of Streptococcus mitior and Streptococcus salivarius were isolated from four subjects and were incubated with homologous and heterologous whole clarified saliva. Supernatants of bacterially absorbed and control saliva were analysed in tandem crossed immunoelectrophoresis (TCIE) against a rabbit anti-human saliva antiserum. 6-8 antigens were detected. After incubation with the bacteria, the saliva supernatants showed a decrease in the number of antigens detectable by TCIE. For all four salivas tested, the homologous group of strains absorbed fewer antigens. Some of the antigens could be eluted from bacterial pellets with 1 M NaCl after repeated washings in PBS. Epithelial cells also absorbed fewer antigens from homologous than heterologous saliva.

Key words: epithelial cells, host specificity, interactions, indigenous bacteria, oral streptococci, salivary antigens.

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INTRODUCTION

Oral bacteria and hydroxyapatite can interact with various salivary molecules such as high molecular weight agglutinins (1-5), blood group reactive substances (6, 7), secretory IgA (8-12), lysozyme (13), fibronectin (14) and β_2 -microglobulin (15, 16). Whether the binding of a particular molecule favours or impairs bacterial colonization in the mouth have been discussed at length (17-19). A dual function of the different salivary components has been suggested. Thus, in some instances, high salivary concentrations of bacterial binding and agglutinating molecules probably impair colonization. Low concentrations may increase colonization if the substances are also present in the pellicle or on mucosal surfaces (5, 20). However, the bacteria - host surface interactions in the mouth are not easily explained by single molecular interactions. Complex interactions between the specific bacterial profile of adhesins and the host tissue profile of ligands, also modified by salivary molecules, are believed to determine the selective colonization abilities of bacteria (17-19). Thus, whereas measuring the binding and the effect of the binding of a component to a particular bacterium may provide very important information about the specificity, it is difficult to explain the overall colonization process by evoking concepts of single molecule interactions.

The aim of this paper was to measure bacterial binding of several salivary components simultaneously, thus trying to provide the basis for further studies into whether bacterial binding of salivary molecules in general seems to be related to oral colonization in vivo by a particular organism.

MATERIAL AND METHODS

Bacterial strains

Fresh strains of oral streptococci were isolated by sampling with a sterile cotton swab from the tongue and buccal mucosa of four subjects (2 males, subjects A and B and 2 females, subjects C and D, aged 25-35), and cultivated on Mitis-Salivarius agar (MS) (Difco, Detroit, USA). After 48 hours' incubation at 37°C, in an atmosphere of 95% N₂ and 5% CO₂, a total of 20 colonies resembling Strepto-

coccus salivarius and Streptococcus mitior were transferred to new MS agar plates and further to Brain Heart Infusion medium (BHI, Difco). After another incubation for 48 hours as above, bacteria were harvested by centrifugation at 2000 x g and washed twice with BHI supplemented with 15% glycerol. The pellet was resuspended in BHI medium with 15% glycerol and 50% sterile blood and mixed with glass beads. Excess medium was removed and the coated glass beads were stored frozen at -20°C.

For each experiment one bead was inoculated in a starter culture consisting of 10 ml of dialyzed yeast extract medium (21) and incubated over night as above. An aliquot of the starter culture was inoculated into 10x10 ml culture tubes of dialyzed yeast extract medium. The cultures were grown over night and the bacteria were harvested by centrifugation at 2000 x g. The pellet was washed twice in 0.01 M phosphate buffered saline (0.15 M NaCl, pH 7.2, with 0.02% NaN₂) and the wet pellet weight was determined.

Epithelial cells

Buccal epithelial cells from four subjects; subject A as above and 2 additional males (E, F), and one additional female (G), (aged 22-40) were collected by gently rubbing the cheek mucosa with a wooden stick. The stick was rotated in 10 ml of PBS and the procedure was repeated until a dens suspension of epithelial cells was acquired. The cells were centrifuged at 200 x g for 10 min. and washed twice in 10 ml of PBS. The wet pellet weight was determined.

Hydroxyapatite

Commercially available spherical hydroxyapatite (HA) (Merck, 80-200 mesh) was washed twice in PBS, and the wet weight was determined.

Saliva

Paraffin stimulated whole saliva from the test persons was collected at the same occasion as the bacteria and was clarified by centrifugation at 2000 x g and frozen in 0.5 ml portions at -20°C. Epithelial cells and saliva were collected at different occasions.

Absorption of saliva

To the wet pellets of bacteria and hydroxyapatite, equal weights of clarified saliva from subjects A, B, C and D were added respectively. As control, saliva was added to empty tubes. The suspensions were mixed, and after 60 minutes at room temperature, the suspensions were centrifuged at 2000 x g for 20 min. Supernatants were analysed by immunoelectrophoresis. Wet pellets of epithelial cells from subjects A, E, F and G were treated in the same way. To try to elute absorbed salivary components from bacterial cells, some bacterial pellets were washed twice with 2 ml PBS followed by 1 M NaCl in distilled water at the same volume as the saliva. The suspensions were mixed and after 10 minutes they were centrifuged and the 1 M NaCl supernatants were analysed.

Rabbit anti-human saliva antiserum (RAS)

One rabbit was given intramuscular injections of 0,5-1 ml of 10 times concentrated saliva from subject A with Freund's incomplete adjuvant. Injections were repeated weekly for 4 weeks. After another period of one week, the rabbit was bled. Whole antiserum was prepared and frozen at -20°C in 1 ml vials.

Tandem crossed immunoelectrophoresis (TCIE) (22)

First dimension. 1% agarose gel (HSA, lot 898, Litex, Denmark) in Tris-barbital buffer (pH 8.6; 0.007 M TRIS, 0.024 M barbital, 0.032M calcium lactate and 0.02% Na_3N) was cast on GelBond films (FMC Corporation, Rochland, Maine, USA, 8x22 cm) and pairs of wells with 3.5 mm diameter were punched approximately 4 mm apart. The distance between each pair of wells was 2.5 cm, and a total of 7 pairs of holes were punched in each gel. 16 μl of absorbed saliva supernatants were applied in the left well and 16 μl of unabsorbed saliva in the right well. After the samples had diffused into the gel, the wells were filled with agarose and a voltage of 7.5 v/cm were applied for approximately 30 min. until a Bromphenol blue indicator had migrated 1.5 cm.

Second dimension. A basin (22x3 cm) was then cut 1.5 cm from the row of sample wells, and agarose gel containing 3% RAS was added. A voltage of 1.5 V/cm was applied perpendicular to the first dimension for 18 hours. Gels were washed twice in 0.15M NaCl and in distilled water, dried, stained with 0.5 % Coomassie Brilliant Blue R 250 (22) and photographed.

Rating of precipitates

The heights of the precipitates formed by absorbed and unabsorbed saliva were compared. The decrease of the height of a precipitate in absorbed saliva supernatants was recorded. The number of decreased precipitates was expressed as percentage of the total number of precipitates in each saliva-bacteria combination. Median values for all strains from one subject were calculated for each saliva.

RESULTS

Detectable antigens in unabsorbed saliva

Whole saliva gave six to eight distinct precipitates against 3 % RAS. Figure 1 illustrates an example of a TCIE gel containing 8 distinguishable antigen precipitates. The left and the right wells contained untreated saliva from subject A, the donor of saliva for immunization of the rabbit. The eight precipitates (1-8) formed showed identity of antigens in both wells, either as two joined peaks (precipitate 1) or as a wide peak (precipitate 2).

As expected the untreated saliva from the four subjects differed. Figure 2 illustrates a comparison between saliva A (to the right) and saliva B (to the left). The antigen corresponding to precipitate 1 was in higher concentration in saliva B, and precipitate 2 was in lower concentration. The antigen corresponding to precipitate 7 was not present in saliva B. The numbers of antigens detected in saliva from all the subjects (saliva A-D) are presented in Fig. 5.

Interactions between saliva and bacteria

A total of 76 supernatants of absorbed saliva from saliva-bacteria mixtures were analysed. Fig. 3 shows the supernatant of saliva B which had been incubated with a homologous strain of S. mitior in the left well and the corresponding untreated saliva to the right. Precipitates 1, 2 and 8 were clearly reduced in height to the left, while the symmetric precipitates 3, 4 and 5 were not. Fig. 4 shows saliva A supernatant after incubation with the same strain as in Fig. 3. The absorbed saliva, to the left, showed a reduction of antigen concentrations corresponding to precipitates 1, 2, 4, 7 and 8. (Cf Fig. 1 showing untreated saliva A in both wells.)

Between 0 and 7 salivary antigens were beyond detection or decreased in concentration after incubation with bacteria. The antigen corresponding to precipitate 2 was reduced in 70 out of 76 assays. In a few instances, the antigens gave less distinct and wider precipitates after absorption.

For each saliva, the percentage of antigens with diminished concentration was calculated for the homologous and heterologous groups of strains respectively. Fig. 5 shows that the homologous groups of strains had less of an antigen-absorbing effect ($p < 0.05$, two-tailed, Wilcoxon's rank sum test).

The 1 M NaCl supernatants from washed bacterial pellets were analysed for the presence of eluted salivary antigens. Fig. 6 shows saliva supernatant from a mixture with an S. mitior strain (left) and corresponding untreated saliva (right). Antigens corresponding to precipitates indicated by arrows were clearly at lower concentration in the treated saliva. In the supernatant of the 1 M NaCl eluate of the pellet (Fig. 7), 2 precipitates were identified (left), which showed identity with antigens in untreated saliva (right).

Interactions between saliva and epithelial cells and HA

Fig. 8 shows saliva A supernatant after incubation with heterologous epithelial cells. Precipitates 1, 2 and 8 were reduced in height. Absorption with epithelial cells decreased the concentration of 2 to 4 antigens. Fig. 9 shows the percentage of antigens which were reduced in concentration for all saliva-epithelial cell combinations. Again, homologous combinations of saliva and epithelial cells showed a lower percentage ($p < 0.02$ two-tailed Wilcoxon's rank sum test).

Incubation of saliva with HA reduced all precipitates. Fig. 10 shows saliva D incubated with HA to the left and untreated saliva to the right.

DISCUSSION

It is very doubtful that oral bacteria transmitted between individuals or between individual sites within the mouth are ever free of adsorbed salivary molecules. Surely these bound molecules must

influence the outcome of implantation and tissue distribution. As a beginning of studies to determine the importance of inter-individual differences of salivary molecules for colonization by the indigenous flora, this investigation has indicated that bacterial and epithelial cell interactions with saliva seem to show a degree of host specificity. Saliva always showed fewer detected interactions with the homologous than with the heterologous groups of bacterial strains and epithelial cells. All salivary antigens showed high affinity for HA. The detected antigens represent what is probably a limited set of salivary substances.

When comparing saliva supernatants which had been mixed with the absorbents and those of control saliva, one or more antigens were always present at the same concentrations, indicating that dilution of the samples was not of significance, at least for testing bacteria and epithelial cells. However, HA greatly reduced the concentration of all antigens and control as above was not possible. In a recent study by RÖLLA and co-workers (23), rabbits were immunized with saliva-coated HA and 5-8 antigens were detected, indicating that HA readily adsorbs several salivary antigens. Interestingly, a similar number of antigens was detected in the present study.

The apparent reluctance of interactions between saliva and homologous epithelial cells might partly be explained by presence of salivary molecules on their surfaces also after repeated washings. Epithelial cells might also carry antigens similar to salivary substances (7). This might have inhibited binding of test saliva.

Bacteria can reduce the concentration of salivary antigens either by adsorption (8) or by degradation (24). Since the assays were carried out at room temperature for not more than one hour, with bacteria washed several times in buffers containing sodium azide, the mode of interaction was probably mostly adsorption. Also, it was possible to elute some of the antigens from washed bacterial pellets with sodium chloride. However, the degradation of salivary substances cannot be excluded, since in some assays a slight change of the electrophoretic appearance of antigens in bacteria-treated saliva was seen.

Some antigens, as for example antigens 1, 2 and 8 (Figs 3, 4 and 8), were bound by bacteria, epithelial cells and HA. The common pattern of reactions, might rise the question whether these antigens could be of a particular interest for the saliva-mediated interactions between bacteria and host surfaces. The possible importance of these antigens needs to be further investigated.

The major original finding of this study was that saliva from the subjects tested always showed the least number of interactions with the homologous group of bacterial strains. This appears to contradict the finding by ØRSTAVIK et al (25) that bacteria colonize homologous pellicles better than heterologous ones, a finding that might favour the idea that binding of several salivary components are advantageous for colonization. However, data summarized by GIBBONS (17), VAN HOUTE (18) and TABAK et al (19) suggest that binding of salivary substances to bacteria might have a dual effect on their colonization abilities. The binding of a selected combination of substances, preferably present on intra-oral surfaces, are likely to promote initial attachment of bacteria. Bacterial binding of soluble substances may be to their disadvantage for attachment, through masking of adhesins, change of important physicochemical surface properties and agglutination into masses more easily removed from the mouth. TABAK et al. (19) proposed that a microheterogeneity, possibly individual, among salivary substances might provide a "zip-code" for clearance or attachment of specific microorganisms. Thus, the binding of selected substances and the apparant avoidance of interaction with others as seen in this study, might be of importance for bacterial colonization. It should follow that bacteria may recognize discrete inter-individual differences in at least some salivary antigens. In fact, from other areas it has been reported that serological variants of strains of Escherishia coli can interact with jejunal brush borders in piglets with a specific phenotype (26). Several salivary components show inter-subject differences (27), and the aquired pellicle show inter-individual differences (28). Indeed, ØRSTAVIK et al. (25) suggested that "it was tempting to postulate" that the indigenous oral bacteria were more or less adapted to the homologous oral milieu, concerning their binding to homologous and heterologous pellicles.

The data from this paper indicate that homologous and heterologous groups of bacteria interact differently with six to eight detected antigens in host saliva. These observations do not necessarily imply that such differences are prerequisites to colonization or an expression of adaptation. However, they certainly can provide a basis for further investigations on the importance of bacterial binding of several salivary molecules and the significance of possible individual host differences of these molecules.

REFERENCES

1. ERICSON TH, PRUITT K, WEDEL H. The reaction of salivary substances with bacteria. J Oral Pathol 1975; 4:307-23.
2. GIBBONS RJ, SPINELL DM. Salivary-induced aggregation of plaque bacteria. "Dental Plaque" McHugh, Livingstone, Edinburgh, 1970; 207-215.
3. HAY DI, GIBBONS RJ, SPINELL DM. Characteristics of some high molecular weight constituents with bacterial aggregating activity from whole saliva and dental plaque. Caries Res 1971; 5:111-23.
4. KASHKET S, DONALDSON CG. Saliva-induced aggregation of oral streptococci. J Bacteriol 1972; 112:1127-33.
5. MAGNUSSON I, ERICSON TH. Effect of salivary agglutinins on reactions between hydroxyapatite and a serotype c strain of Streptococcus mutans. Caries Res 1976; 10:273-86.
6. GIBBONS RJ, QUERSHI JV. Selective binding of blood group-reactive salivary mucins by Streptococcus mutans and other oral organisms. Infect Immun 1978; 22:665-71.
7. WILLIAMS RC, GIBBONS RJ. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect Immun 1975; 11:711-18.
8. BRANDTZAEG P, FJELLANGER I, GJERULDSSEN ST. Adsorption of immunoglobulin A onto oral bacteria in vivo. J Bacteriol 1968; 96:242-9.
9. KILIAN M, ROLAND K, MESTECKY J. Interference of secretory immunoglobulin A with sorption of oral bacteria to hydroxyapatite. Infect Immun 1981; 31:943-51.
10. LILJEMARK WF, BLOOMQUIST CG, OFSTEHAGE JC. Aggregation and adherence of Streptococcus sanguis: role of human salivary immunoglobulin A. Infect Immun 1979; 26:1104-10 .
11. OLSSON J, BRATHALL D, CARLÉN A. Association between bacterial agglutinins and immunoglobulin A in human saliva. Acta Odontol Scand 1981; 39:61-6.
12. WILLIAMS RC, GIBBONS RJ. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science 1972; 177:697-9.
13. POLLOCK JJ, IACONO VJ, GOODMAN BICKER H, et al. The binding, aggregation and lytic properties of lysozyme. Eds. Stiles, Loesche and O'Brien, Proceedings "Microbial Aspects of Dental Caries" Sp Supp Microbiology Abstracts 1976; II:325-52.
14. BABU JP, SIMPSON WA, COURTNEY HS, BEACHY EH. Interaction of human plasma fibronectin with cariogenic and non-cariogenic oral streptococci. Infect and Immun 1983; 41:162-8.

15. ERICSON D, BJÖRCK L, KRONVALL G. Further characteristics of β_2 -microglobulin binding to oral streptococci. *Infect Immun* 1980; 30:117-24.
16. ERICSON D, BRATTHALL D, BJÖRCK L, MYHRE E, KRONVALL G. Interactions between human serum proteins and oral streptococci reveal occurrence of receptors for aggregated β_2 -microglobulin. *Infect Immun* 1979; 25:279-83.
17. GIBBONS RJ. Microbial ecology. Adherent interactions which may affect microbial ecology in the mouth. *J Dent Res* 1984; 63:378-85.
18. HOUTE J, VAN. Are the concepts proposed for dental plaque formation valid? In: B. Guggenheim, ed. *Cariology Today*. Int Congr, Zürich, 1983. Basel, Karger 1984. 182-90.
19. TABAK LA, LEVINE MJ, MANDEL ID, ELLISON SA. Role of salivary mucins in the protection of the oral cavity. *J Oral Pathol* 1982; 11:1-17.
20. LILJEMARK WF, BLOOMQUIST CG, GERMAINE GR. Effect of bacterial aggregation on the adherence of oral streptococci to hydroxyapatite. *Infect Immun* 1981; 31:935-41.
21. CARLSSON J, NEWBRUN E, KRASSE B. Purification and properties of dextransucrase from Streptococcus sanguis. *Arch Oral Biol* 1969; 14:469-78.
22. KROLL J. Tandem crossed immunoelectrophoresis. In: Axelsen NH, Kroll J, Weehe B, ed. *A manual of quantitative immunoelectrophoresis. Methods and applications*. Scand J Immunol 1973; 2 suppl 1 57-9.
23. RÖLLA G, CIARDI JE, BOWEN WH. Identification of IgA, IgG, lysozyme, albumin, α -amylase and glucosyltransferase in the protein layer adsorbed to hydroxyapatite from whole saliva. *Scand J Dent Res* 1983; 91:186-90.
24. KILIAN M, HOLMGREN K. Ecology and nature of immunoglobulin A1 protease-producing streptococci in the human oral cavity and pharynx. *Infect and Immun* 1981; 31:868-73.
25. ØRSTAVIK J, ØRSTAVIK D, KOMMISAR J. Preferential affinity of oral bacteria for homologous salivary films on dental materials. *Acta Odontol Scand* 1982; 40:49-56.
26. BIJLSMA IGW, NIJS A DE, MEER C VAN DER, FRIK JF. Different pig phenotypes affect adherence of *Escherichia coli* to jejunal brush borders by K88ab, K88ac, or K88ad antigen. *Infect Immun* 1982; 37:891-4.
27. ARGLEBE C. Biochemistry of human saliva. In: Pfaltz CR ed. *Sialadenosis and sialadenitis*. Adv Oto-Rhino-Laryng, Basel, Karger 1981; 26:97-234.
28. KRAUS FW, ØRSTAVIK D, HURST DC, COOK CH. The acquired pellicle: Variability and subject-dependence of specific proteins. *J Oral Pathol*. 1973; 2:165-173.

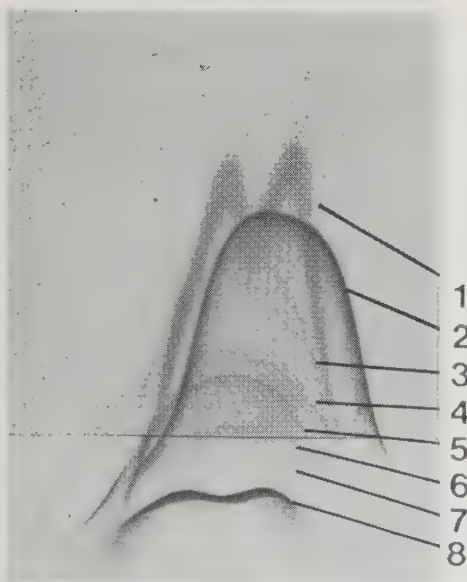


FIG 1. TCIE of saliva from subject A in both wells (wells not seen). Antigens are numbered (1-8).

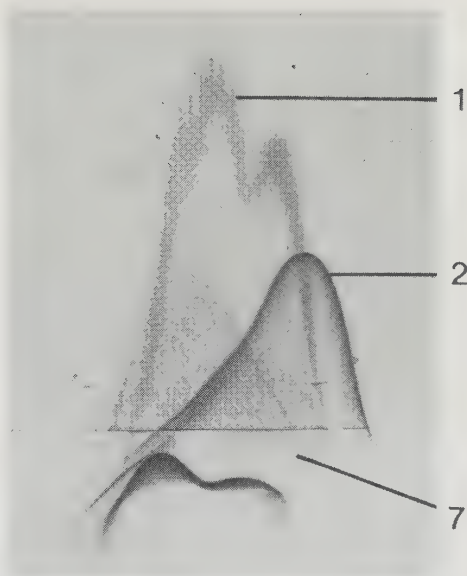


FIG 2. TCIE of saliva from subject A in the right well and subject B in the left well. Some antigens (1, 2, 7) with marked concentration differences in the two salivas are indicated.

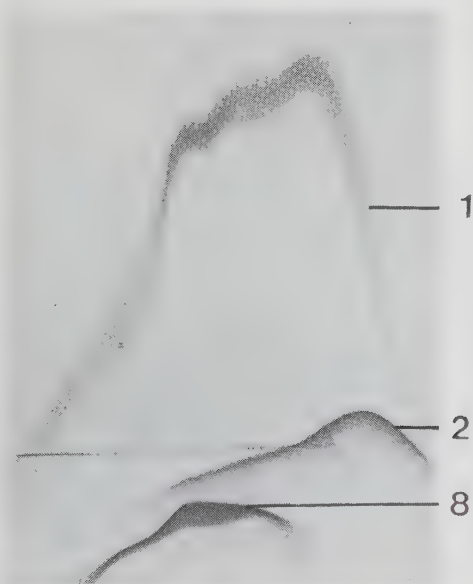


FIG 3. TCIE of saliva from subject B in the right well and the supernatant from saliva B after incubation with a homologous S. mitior strain in the left well.

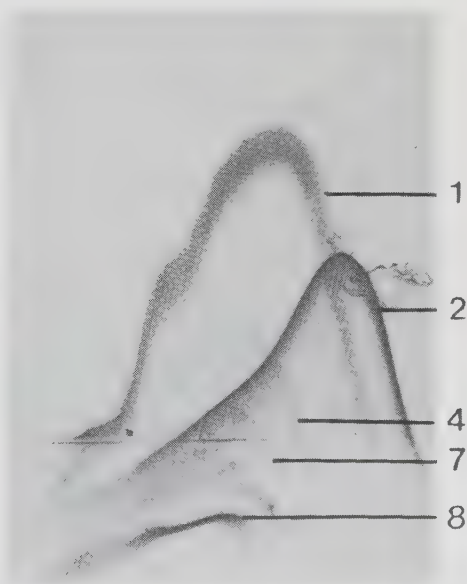


FIG 4. TCIE of saliva from subject A in the right well and the supernatant from saliva A after incubation with the same strain as in FIG 3 in the left well.

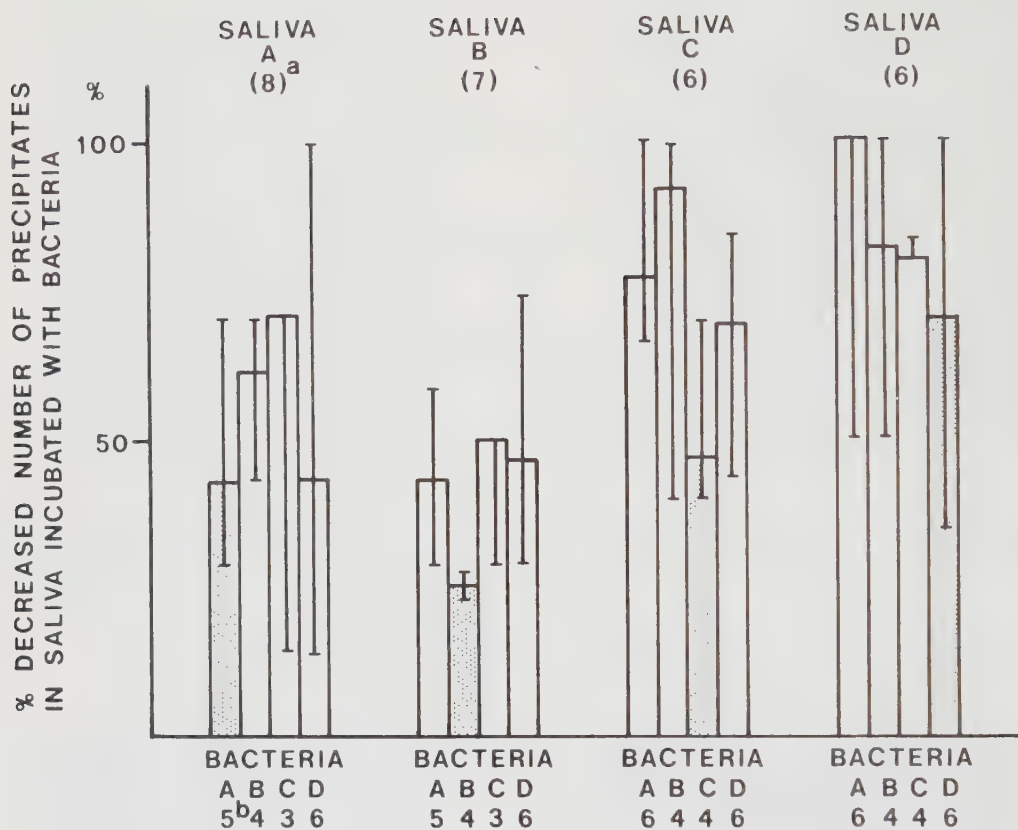


FIG 5. Percent of decreased number of antigens in saliva supernatants from subject A, B, C and D after incubation with homologous and heterologous strains. Median values and ranges are presented.

a: number of antigens detected in saliva

b: number of strains tested.

Grey columns represent homologous strains.

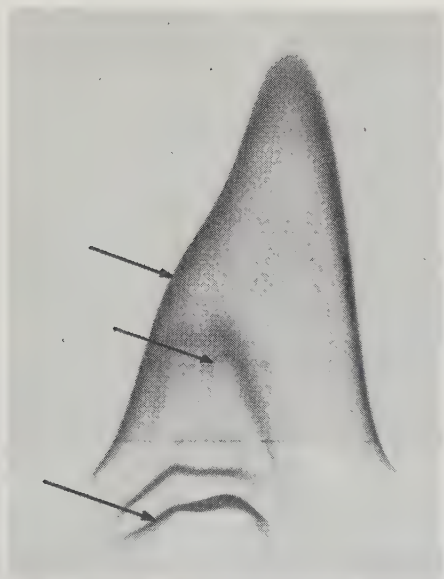


FIG 6. TCIE of saliva supernatant after incubation with a *S. mitior* strain in the left well and untreated saliva in the right well. Antigens with a lower concentration in bacteria-treated saliva are indicated.

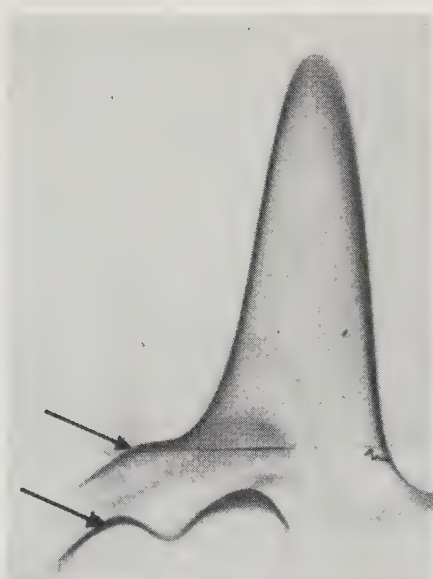


FIG 7. TCIE of 1 M NaCl eluate of the washed bacterial pellet from FIG 6 to the left as compared to untreated saliva to the right. Antigens found in the eluate are indicated.

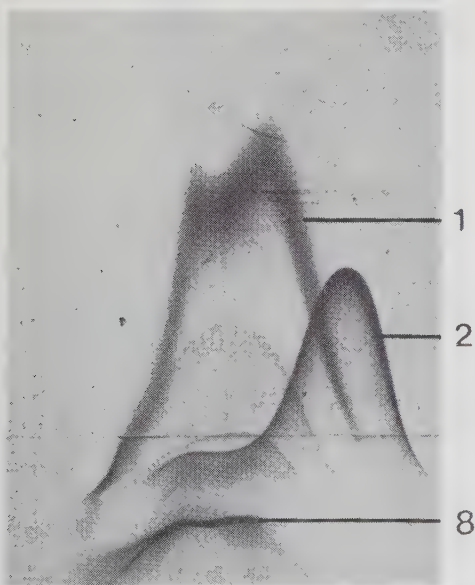


FIG 8. TCIE of saliva supernatant from subject A after incubation with heterologous epithelial cells in the left well. Untreated saliva in the right well.

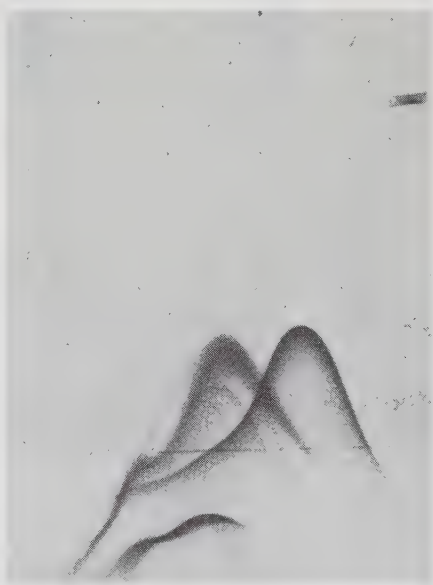


FIG 10. TCIE of saliva from subject D incubated with HA in the left well and untreated saliva in the right well.

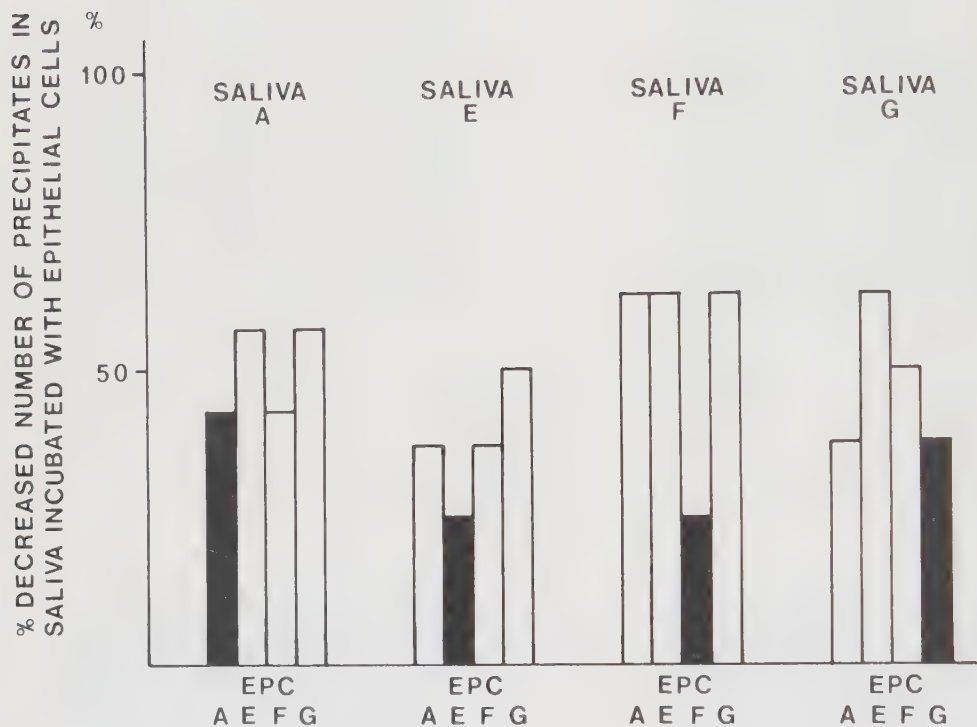


FIG 9. Percent of decreased number of antigens in saliva supernatants from subjects A, E, F and G after incubation with homologous and heterologous epithelial cells.

Black columns represent homologous cells.

